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SAP HYDRAULICS

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(WITH ELEVEN FIGURES)

Introduction

In animals, the chief function of gas exchange in the lungs is to supply oxygen for, and to remove the products of respiration, whereas in plants the chief function of gas exchange in the leaves is to supply carbon dioxide for photosynthesis. Since the protoplasm of the plant or animal body contains a high percentage of water, it is inevitable that both of these processes are accompanied by a loss of water in the form of gas, *i.e.*, water vapor. However, since there is seven hundred times as much oxygen as carbon dioxide in the air (**96**), which is the normal environment of plants and animals, the latter can obtain that which they need from a smaller volume of air than can plants. Consequently, being necessarily exposed to a larger volume of air, a greater amount of water is evaporated from plants than from animals, and that which is of minor importance in animal physiology becomes one of the major problems of plant physiology.

To replenish the enormous water loss (**19, 74**) accompanying gas exchange in the leaves necessitates the movement of water through the plant body from the roots to the leaves. Aside from preventing the desiccation of the plant, this water movement and loss may be of minor benefit to the plant (**110, 9, 54, 28, 88**) in its life processes; nevertheless, it is of sufficient magnitude to arouse the interest of the observer. The warm dry air prevalent during summer in California provides a situation particularly favorable for the observation of these phenomena. If any one phase be studied alone, without due consideration of its relationships to plant physiology as a whole, erroneous conclusions may be reached such as those implied by references in the literature (**93, 27**) to the transpiring "power" of plants. Consequently, the writer's observations of the transpiration stream have included studies of the entrance of the water into the roots, the passage of that water through the plant, and its exit through the leaves. This inclu-

sive subject I have termed "sap hydraulics," and the present study is the continuation of a previous one bearing the same title (**109**).

COPELAND (**22**) gives a review of the early literature on this subject, followed by a bibliography including 174 titles. BURGERSTEIN (**20**) presents a bibliography with nearly 1,200 titles up to April, 1925. Although but a portion of the total available, the writer has examined an additional hundred pertinent references appearing since that date. MILLER (**69**) also presents excellent bibliographies. It is obvious that any attempt at a complete review of the literature on this subject would require a monograph.

A consideration of plant anatomy and physiology as a background for sap hydraulics shows that the transpiration stream moves through a system of vessels, the walls of which are wet by water continuous with that which it is conducting. This xylem is surrounded on all sides and terminally by living cells. Conduction of nutrient materials takes place through the phloem by diffusion through the protoplasm, particularly of the sieve tubes, aided by protoplasmic streaming; or by diffusion through the cell walls, aided by concentration gradients and mass flow as recently proposed by CRAFTS (**27**); or by a combination of the two.

1. Entrance of water into roots

The region of entrance of water into the root has been determined by COUPIN (**24, 25, 26**), PRIESTLEY *et al.* (**77, 78, 79, 80, 81, 82, 90, 91**) and POPESCO (**76**). They are in agreement that the region of maximum absorption under conditions of plentiful water supply lies below the region of root hairs and that the latter are not indispensable, although PRIESTLEY points out that, under conditions of deficient soil moisture, they greatly increase the surface area of the root in contact with the soil. Since it is now known (**21, 49, 57, 106**) that there is but little movement of moisture in the soil under such conditions, and that the roots have to grow to it, the significance of PRIESTLEY's observation is readily appreciated.

The upper limit of the absorptive area is determined by the deposition of waxy materials in the cell walls of the endodermis. The lower limit may be the terminal embryonic region. PRIESTLEY's observation that this region offers a continuity with the protoplasm of the endodermis is morphologically correct; but his contention that no water is absorbed by this region is based upon observations, the insufficiency of which is shown by his later work.

In his earlier work PRIESTLEY made use of dyes in his determinations. He now emphasizes his agreement with other workers (**4, 5, 14**) that water entry and salt entry are distinct processes, using, as evidence, some of the very dyes from both the acid and basic groups which he had used in attempting to delimit the zone of water entry.

The writer has performed experiments with four of the dyes used by him, namely, methyl green, acid green, malachite green, and neutral red. These dyes were added to dilute lithium nitrate solution as a medium for water cultures of corn, sunflower, buckeye, and willow plants. In every instance the lithium passed up the stem as shown by spectroscopic test, while not the slightest trace of the dye could be found. These experiments merely emphasize the independence of movement of the water and the dye dissolved in the water, as elucidated by SACHS (**85**). The same conclusion

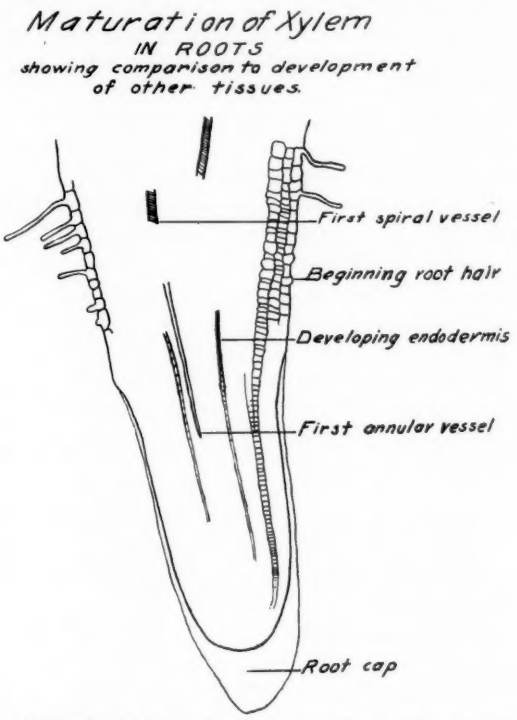


FIG. 1. Longitudinal section of *Cosmos* sp. root tip showing relationship of maturing xylem vessels to other tissues. × 70.

was reached by the use of a fifth of these dyes, eosin, on potted plants of eucalyptus. The eosin diffused into the roots so slowly, even in 24 hours, that it did not appear in the xylem at all.

POPESCO's conclusions are therefore to be preferred, namely, that some water may be absorbed by the embryonic region of the root for growth at that point. Absorption of water for the vessels takes place between the

level at which the differentiation of vessels begins up to the level at which the endodermis is suberized, with a maximum at the lower limit of the region. Since spiral tracheae can be found within 1 mm. of the root tip (fig. 1), and COUPIN immersed his root tips 2 to 3 mm., his conclusions are not in discord with those of POPESCO.

Considering next the mechanism of water entry: under conditions of rapid transpiration or deficient soil moisture, where the water in the vessels is under tension, that tension may be transmitted through the tissues of the root to the soil moisture, drawing the water into the root without the intervention of any osmotic phenomena. The fact that passageway cells in the endodermis opposite the xylem points are at the level of developed root hairs is of significance.

Under conditions of plentiful water supply, PRIESTLEY (*loc. cit.*) explains water entry and root pressure by an osmotic gradient between the soil solution and the inside of the endodermis, set up by the liberation of solutes when the cell membranes of the differentiating xylem break down and become permeable. This would necessitate active growth for the manifestation of root pressure, and is in accord with the observations presented herewith. However, in the case of *Colocasia* at least (FLOOD **38**), this would necessitate the assumption of resorption of the solutes. After the protest of BLACKMAN (**11**), PRIESTLEY presented evidence to justify this assumption. An alternative mechanism will be presented which does not necessitate this assumption and which is in better accord with root anatomy.

2. Guttation experiments showing correlation between active growth and root pressure

In a previous study on sap hydraulics, I have shown that guttation is not due to any local glandular action, but rather to hydrostatic pressure arising within the roots. This is in accord with the observations of FLOOD (**38**) and LEPESCHKIN (**55**).

In the present study use has been made of a large abandoned cistern located under the experimental glass-house of the Stanford Botany department. The natural environment provided by this cistern is such as to make artificial control of conditions unnecessary. Seepage water affords a constant and practically saturated atmosphere. The temperature in the absence of a light is constant, over a considerable time.

A series of five experiments was run on seedling wheat and barley, and one on nasturtium. It was necessary to use powdered sulphur on the surface of the soil in order to keep down the growth of fungi.

EXPERIMENT 1, started February 11, 1931; temperature 20° C.; no light.

A pot of seedling wheat was placed in the cistern. Water of guttation was wiped off the leaves for the next five days. There was no guttation on

the sixth day. Placing the pot in a pan of water produced no guttation, showing that the amount of water at the roots was not a limiting factor. On the seventh day the pot was placed under a bell jar and removed to the basement, thereby subjecting the plants to a drop in temperature of 5.5° C. This produced but very slight guttation.

By this time the plants were pale green in color, due to growth in the dark. On the evening of the eighth day, the bell jar was placed under a strong light. The following morning there was some injury from the heat of the light and the plants were returned to the cool basement. The next morning there was copious guttation, even on the tips of leaves completely desiccated from heat injury.

EXPERIMENT 2, started February 11, 1931; temperature 20° C.; no light.

Wheat seeds were germinated in a pot of soil under a bell jar with an excess of water. There was slight guttation on the tips of the seedlings. On the third day following germination, the lid was removed from the cistern for several hours, permitting sunlight to enter. The following day all of the seedlings showed marked elongation and active guttation.

EXPERIMENT 3, started April 27, 1931; temperature 21° C.; no light.

A pot of seedling wheat was placed under a bell jar, with an excess of water. Guttation had ceased by the seventh day. The plants were again a pale green.

At 10:30 on the morning of the eighth day, a 200-watt nitrogen-filled Mazda lamp was suspended about a meter above the bell jar and turned on for just five minutes. Four hours later, guttation had been resumed by one of the plants. The light was then turned on for a full hour, producing copious guttation against a rise of 0.5° in temperature. This observation of guttation under a rising temperature led to the following experiment.

EXPERIMENT 4, started May 11, 1931; temperature 21° C.

A pot of barley seedlings was placed under a bell jar with an excess of water at 11:00 A. M. At 1:30 P. M. guttation was visible and the 200-watt light was turned on. Four hours later the temperature had risen 1°. All of the plants were guttating freely.

By the fifth day the temperature had risen to 23.5° C. The plants had been guttating continuously under the rising temperature. On the sixth day the temperature had risen to 24° C. and guttation had ceased, probably owing to decreased humidity at the higher temperature. That night the light was turned off and the cover removed from the cistern. This lowered the temperature again to 21° C. The following morning the light was turned on once more and the plants resumed guttation. In this manner the plants were made to guttate almost continuously for two weeks.

EXPERIMENT 5, started May 25, 1931; temperature 21° C.

A pot of barley seedlings which were grown in a dark room until guttation had ceased, even with an excess of water at the roots, was placed in the cistern under a bell jar with an excess of water. These seedlings had made good growth and showed guttation on two or three blades only when placed under the bell jar. The light was then turned on, but no additional guttation could be produced. Even placing the bell jar with the plants in an ice-box did not produce guttation.

This experiment was repeated with a mature water culture of wheat which had developed a head. No guttation could be produced by illumination, decreased temperature, or increased humidity of the atmosphere.

EXPERIMENT 6. A potted nasturtium which had ceased active growth was placed under a bell jar with an excess of water. The nasturtium is a standard plant for experiments on guttation; nevertheless this plant did not guttate even though the conditions were such as to cause condensation of water in the bell jar. In another instance such a plant was caused to resume guttation by repotting and allowing new root growth to occur.

CONCLUSIONS

These experiments show that even under conditions of lowered temperature, high relative humidity, and an excess of water in the soil—all of which are favorable to guttation,—plants will not show this phenomenon unless actively growing. If actively growing, then, with an adequate water supply and a high relative humidity, plants will continue to guttate even in a rising temperature.

Since guttation is a manifestation of root pressure, the latter is produced only under conditions of active growth.

3. Alternative hypothesis for water entry into roots in absence of tension forces

Knowing that root pressure is developed only under conditions of active growth, a review of root anatomy leads to the following explanation of water entry in the absence of tension forces from transpiration, extending the mechanism of PFEFFER (**75**), BLACKMAN (**11**), and MÜNCH (**71**).

The root is terminated by a region of actively growing embryonic cells. In the process of growth, cells absorb large amounts of water. Therefore the cells of the root tip will absorb water directly from the soil for this purpose; the cells of the root cap beyond the embryonic region have no easier means of obtaining it.

The enlarging cells above the embryonic region may also absorb water for growth directly from the soil. In addition to water, however, these cells and the terminal meristem itself need large amounts of nutrients which are brought in solution through the phloem. These solutes, by metabolism,

will be incorporated into the substance of the cell. The excess solvent, after providing water for growth, will be expelled by the path of least resistance. The active growing point is on the inside of the root and the maturing vessels are also on the inside of the root; consequently the excess water will be expelled into the vessels of the xylem. The endodermal sheath will tend to prevent the escape of any water.

In the region of the root where maturation is taking place, the phloem occupies as much as nine-tenths of the circumference of the stele adjacent to the endodermis (fig. 2). The phloem is carrying, either in solution or

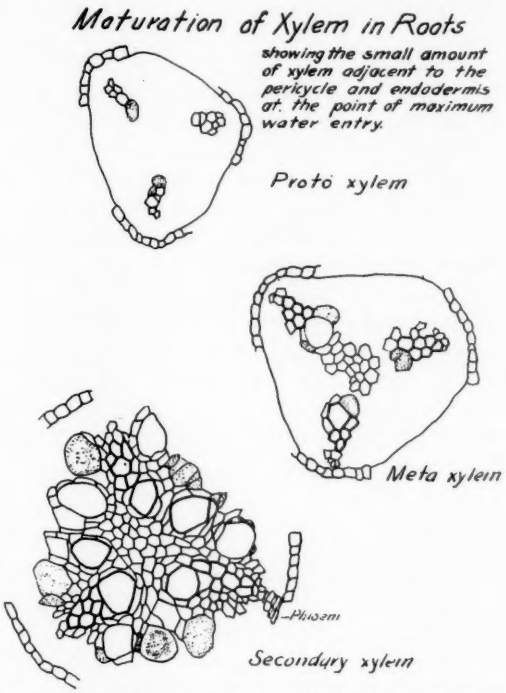

Maturation of Xylem in Roots
showing the small amount of xylem adjacent to the pericycle and endodermis at the point of maximum water entry.
Proto xylem
Meta xylem
Phloem
Secondary xylem

FIG. 2. Transverse section of stele of *Salix lesiolepis* root showing small amount of xylem adjacent to pericycle and endodermis at point of maximum water entry where primary xylem elements are maturing. ×150.

chemical combination, a higher concentration of nutrients (or in other words, a lower concentration of water) than the adjacent tissues. Consequently it is natural that water should be drawn through the endodermis into the phloem. Then, following the process of growth in adjacent maturing cells, or those nearer the growing point, that same water will be expelled

into the xylem as the path of least resistance. Small amounts of solutes may be carried with the water, varying with the protoplasm of the plant in question. In this manner a hydrostatic pressure may be created, giving rise to guttation of water containing only traces of solutes and without invoking any mechanism for resorption.

After tension is created under rapid transpiration, water will be drawn directly into the xylem through the passage-way cells in the endodermis at the xylem points.

This explanation would seem to be in better accord with root anatomy than is that offered by PRIESTLEY.

4. Movement of water through vessels

The foundations for sap hydraulics were laid by HALES (**41**) in 1727. He first called attention to the magnitude of transpiration. He also gave "proof . . . of the serviceableness of the leaves in drawing up the sap" through "perspiration, by the action of warmth" and "the great force with which plants . . . imbibe moisture up their capillary sap vessels," . . . "also of the sap's freely either ascending or descending, as it shall happen to be drawn by the perspiring leaves." "And that plants can plentifully imbibe moisture through their stems and leaves as well as perspire it."

When it was found that capillarity alone was inadequate to account for movement through the xylem, other mechanisms were invoked, such as the alleged lifting power of a Jaminian (**46**) chain of air bubbles, or the pumping effect of living cells, advocated even today by BOSE (**15, 16, 17**).

Although living cells in the leaves may be necessary for transpiration, it has been shown that the actual movement of the water through the xylem vessels constitutes a problem in hydraulics. That living cells along the conducting tract, although they may be of value, are not a necessity, is brought out by the work of STRASBURGER (**100, 101**), OVERTON (**73**), RENNER (**84**), SCHMUCKER (**89**), and MACDOUGAL, OVERTON, and SMITH (**66**).

The simple physical nature of the problem is further brought out by the experiments on reverse flow and absorption by leaves performed by numerous investigators, including HALES (**41**), DANDENO (**30**), WETZEL (**108**), ARNDT (**1**), and HARVEY (**43**). The application of hydraulics is further developed by the correlation of movement through the vessels with POISEUILLE'S (**48**) formula for capillary tubes made by EWART (**37**), and the even closer correlation between rate of flow and vessel diameter for different sizes of vessels made by FURR (**39**).

The search for some simple physical mechanism has revealed but two which have attained general favor with botanists: the imbibition theory of SACHS and the tensile column theory of DIXON and ASKENASY.

SACHS (**85, 86, 87**), following UNGER (**104**), conceived of the sap as imbibed in the cell walls, even as water of crystallization is bound in a crystal. In such circumstances the weight of the water is eliminated as a factor in sap hydraulics. Because SACHS insisted that imbibition was a phenomenon distinct from capillarity, COPELAND (**22**) concluded that, "This robbed the imbibition theory of its foundation in established physics." Both SACHS and COPELAND were correct. LANGMUIR (**52**), followed by MCBAIN *et al.* (**58**) and HARDY (**42**), had not yet developed their theory of sorption now accepted in established physics and confirming SACHS' ideas. It was not because of this, however, but because he thought that the water moved only through the walls of the vessels, that his theory was displaced. Plugging experiments showed that the water moved, for the most part, through the lumen of the vessels.

DIXON (**31, 32, 34, 35**) and ASKENASY (**2, 3**) independently developed a theory of sap hydraulics which had been earlier suggested as a possibility, as related by ASKENASY. They postulate tensile stress generated in the sap at the mesophyll cells of the leaves, transmitted the length of the stem through the cohesive force of water, made possible "only when it is adhering completely to a rigid envelope which confers on the liquid a pseudo-rigidity."

That tension forces exist in the trunks of trees there can be no doubt. That they may amount to several atmospheres is possible. That they are not usually sufficient even to balance the atmosphere and produce a zero pressure is shown by the manometer results obtained by JONES *et al.* (**47**), EWART (**37**), REINDERS (**83**), quoted by OVERTON (**73**) and PRIESTLEY *et al.* (**79**), COPELAND (**23**), HUBER (**44**), and MACDOUGAL (**64**). These works instance greater tensions found at lower levels, which is the reverse of that which should be found in a tensile column. Although he estimated higher tensions, NORDHAUSEN (**72**) reached the same conclusion. Also, a careful examination of MACDOUGAL's figures suggests that higher tensions frequently go with atmospheric conditions favorable to the tree, causing a reduction in transpiration. Such results have caused most of these investigators to question the tensile column theory.

5. Experiments showing need of modifying tensile column theory

In an experiment with *Pittosporum undulatum*, PEIRCE and his students obtained after several hours a rise of 49 cm. of mercury, owing to the tension set up by transpiration. Air then appeared at the lower end of the branch, and the column of mercury fell. This was interpreted as an indication that the xylem walls permit air to penetrate before such tensions are created as would be required in tall trees, under the DIXON-ASKENASY hypothesis. As

a result of this experience PEIRCE expressed his doubts as to the adequacy of the tensile column theory at the Pasadena meeting in 1931.

Working with fragments of xylem from conifers, BAILEY (**8**) found that "the surface tension of sap in the pit membranes of various conifers can be overcome by pressures of less than three atmospheres," and consequently expressed the same doubt.

COPELAND (**22**) reports similar experiments by a number of investigators, all of whom arrived at the same conclusion.

Although the procedure is not new, a number of such experiments were performed with apparatus as illustrated in figure 3, and results obtained

FIG. 3. Apparatus for demonstrating suction tension from transpiration.

which make a significant addition to those already reported.

With *Ricinus communis*, on June 12, under outdoor summer weather, in five minutes a column of mercury was obtained nearly equal to that obtained in the laboratory in a number of hours. The tension was probably greater in the xylem vessels than the 46 cm. of mercury indicated, since the latter was the net result of the water tension and the force with which the air was entering. Also there must have been internal injury from the air passing through the xylem where ordinarily it would have entered and become static. This experiment was repeated on July 3, a day on which the local maximum temperature record of 26 years' standing was officially exceeded. The curves showing the rise of the mercury plotted against time, together with the temperature, relative humidity (from MARVIN'S tables, **67**), and evaporation[1] from a Livingston standard white porous cup atmometer, are shown in figure 4. On an excessively hot, dry day, when we should expect to find a greater tension exhibited by the plant, it is unable to produce the results

[1] Courtesy of Mr. DELZIE DEMAREE.

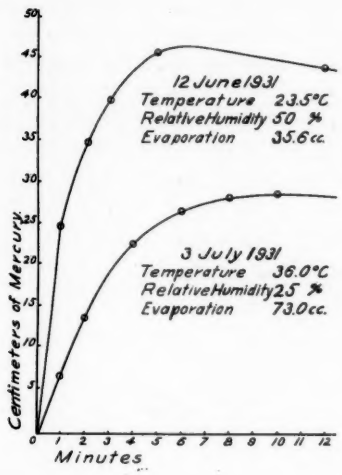


FIG. 4. Record of suction tensions generated by *Ricinus communis* under widely different atmospheric conditions.

obtainable on a more favorable day. MACDOUGAL'S work, previously mentioned, was suggestive of the same thing.

Since there is a higher rate of transpiration on a day when smaller tensile forces are exhibited, some other factors must enter into a consideration of sap hydraulics.

The following experiment is suggestive of an explanation for the mechanism of sap hydraulics. In attempting to demonstrate tensile forces in water, a glass **U**-tube, 14 m. in height, was constructed in a stair-well of the Botany building at Stanford University. The tube was approximately 1.75 mm. inside diameter, and sections were joined with a sleeve of larger tubing held in place by DeKhotinsky cement. At the bottom, as an integral of the tube, was a reservoir with a connection to a second bottle, so that the tube could be filled from the bottom by pressure on the latter, thereby eliminating air bubbles. The top of one arm of the **U**-tube was connected by a stopcock to a vacuum system. It was hoped that the velocity of the water, descending in one arm of the tube and rising in the other, combined with its cohesive properties, would carry it to an elevation greater than the barometric column. Unlike ASKENASY (**3**) and THUT (**102**), under the conditions of this experiment no tensile column was obtained. The water entered the vacuum chamber in spurts, and numerous bubbles formed in the column.

With the aid of a steel piano wire, a thread of white darning cotton (J. & P. Coats 4 ends of 2 ply) was run through the ascending arm of the

U-tube and the experiment repeated. Although the rate of flow was reduced, no bubbles appeared and the flow into the vacuum chamber was uniform, emphasizing the importance of the wettable wall to which DIXON has called attention.

The vacuum chamber was then disconnected and the rate of downward flow due to gravity was measured through the tube with the thread. The results are plotted in figure 5. As the height of the hydrostatic column

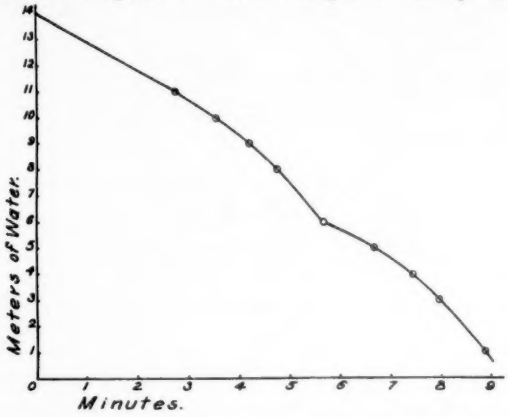


FIG. 5. Downward flow due to gravity, of water through a 1.75 mm. glass tube containing a thread of darning cotton.

decreased the rate of flow increased, showing sorption by the cellulose of the cotton to have been a more important factor than the attraction of gravity. The break in the curve at the height of 6 m. is probably due to some irregularity in the diameter of the tube. Repetition gave duplication.

A cross-section of this thread, when imbedded in paraffin within the tube, occupied 30 per cent. of the lumen of the tube. Planimeter measurements on camera lucida drawings of xylem vessels from twelve or more different plants show that the wall material, considering the middle lamella as the boundary of the lumen, occupies anywhere from 15 to 75 per cent. thereof, the proportion being higher in smaller vessels. These comparisons are presented in figure 6.

This experiment shows that under conditions simulating a xylem vessel, sorption can aid in supporting the weight of the water. In a xylem vessel this would be even more marked, so that any hydrostatic pressure in the tree might be eliminated.

6. Combined tensile fluid-imbibition theory

The forces controlling water movement in the plant are those of osmosis, sorption, or imbibition by cellulose walls and protoplasm, and the cohesive

forces in water itself. A balance is maintained among these forces. SHULL (**95**), in considering this balance, calls attention to the fact that the force of cohesion in water is less "than the adhesion or imbibitional attractions between water and wall substance and protoplasm." VINES (**107**) had previously offered a similar opinion. Because LECLERC DU SABLON (**54**) overemphasized osmosis by living cells in the maintenance of this balance, he

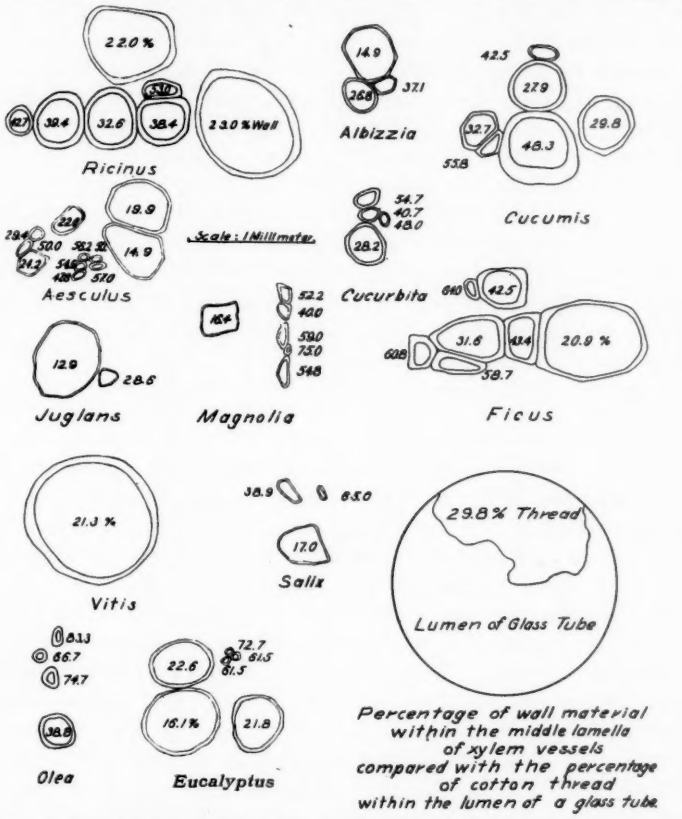


FIG. 6. Percentage of wall material within the middle lamella of xylem vessels compared with percentage of cotton thread within the lumen of a glass tube.

has been classed as a vitalist. His insistence that "la tige supporte suelement le poids de l'ensemble du systeme" is overlooked.

These observations need not detract from the contribution which DIXON and ASKENASY have made to sap hydraulics. On the other hand, their contentions should not be permitted to detract from, nor overshadow, the con-

tributions of SACHS. The modern conception of sorption as given by LANGMUIR (**52**), and developed by HARDY (**42**) and MCBAIN and DAVIES (**58**), weld these two theories together into a harmonious whole which can satisfactorily explain the mechanism of sap hydraulics. LANGMUIR found that sorbed gases are held in a monomolecular film. In the case of liquids, HARDY, MCBAIN, and DAVIES found that there are oriented chains of molecules attached to the monomolecular films and extending the influence of sorption to measurable microscopic distances. The diameter of xylem vessels, which always contain water, as determined by BODE (**12**), agrees with these measurements. Thus it is logical to conclude that the "wires of water" are moved by tensile stress, but supported by sorption.

If to this we add the rôle of metabolic water in repairing broken columns in the xylem vessels, overlooked by BABCOCK (**7**) in his consideration of the subject but developed by MÜNCH (**71**), we shall have removed LIVINGSTON'S (**57**) tentative supposition "that if the taut water mass is ever broken in a vessel segment, it is never replaced." By this addition, the theory of sap hydraulics approaches completion. Since MACDOUGAL (**59, 60, 61, 63**) has shown by dendrographic records that growth in trees takes place at night, such repairs would take place at night, also.

DIXON (**35**) also suggests a zymogenetic secretive function of living cells, to prevent the vessels from clogging.

7. Activating forces in the leaf

VAN TIEGHEM (**103**) conceived of transpiration as "chlorovaporization," a by-product of photosynthesis. From a study of green and etiolated plants, WOODS (**110**) concluded that this process was purely evaporation. "It is evident, therefore, that so-called 'transpiration' is not something which protoplasm *does,* but something which it *resists.*" More recently, IWANOFF and THIELMANN (**45**) showed conclusively that transpiration is not chlorovaporization. They found from 17 to 60 per cent. increase in transpiration on changing from the red-yellow or photosynthetic zone of the spectrum to the blue-violet zone.

DIXON (**31, 32, 33, 34, 35**) has consistently maintained that transpiration is due primarily to secretory action in the cells of the leaves. In the previous study, I showed that water movement in the vessels, in the absence of transpiration, was due to water deficit in cells along the vessels rather than to secretory action; and that DIXON in his experiments did not allow time for a water equilibrium to be established. The comparatively humid climate under which he worked did not sufficiently emphasize the rather long period of time necessary. Further evidence was also presented against secretion through experiments on guttation. This criticism of DIXON has been confirmed by the work of SMITH, DUSTMAN, and SHULL (**97**).

Although designed for another purpose, one of the experiments of BODENBERG (**13**) with lithium nitrate on *Salix* offers similar evidence.

The activating force for the transpiration stream most frequently found in current literature is the evaporation of water from the menisci of submicroscopic pores in the walls of the mesophyll cells of the leaves (**62, 66, 69**). SHULL (**95, 97**) gives a detailed presentation of the propagation of this force into the cell and its conversion into imbibitional and osmotic forces.

Much work has been done by URSPRUNG (**105**) and others (**10, 70**) toward evaluating osmotic pressure and suction tension. This work distinguishes between the suction tension of the cell under normal conditions, due to the net result of turgor pressure outward and wall pressure inward, and the suction tension of the cell under incipient plasmolysis, due entirely to the osmotic pressure of the cell contents. This work does not distinguish between the suction tension or pressure due to osmotic pressure developed by the cell sap and imbibition pressure or tension developed by the protoplasm itself. Of course these three factors of osmosis by the cell sap, imbibition by the cell protoplasm, and elastic tension by the cell wall are constantly undergoing change in a dynamic system such as a living plant cell.

The work of LIVINGSTON (**56**), SHREVE (**92, 94**), BOSWELL (**18**), DUNN *et al.* (**36**), MEYER (**68**), and others (see **6** and **40**), has developed the importance of the latter factor; while KUNKEL (**51**) and LAPICQUE (**53**) would abandon osmosis entirely. In my previous study, on the basis of theoretical physics, evaporation against the greater attraction of large organic molecules constituting protoplasm was suggested as an alternative force for activating the ascent of sap, or, in other words, the force of imbibition. "Consequently in this case, also, it would take more than 536 calories to evaporate one gram of water, the difference being a measure of the work energy available for lifting the tensile columns." The difference referred to is the heat of imbibition readily demonstrable in a Dewar flask.

That evaporation against the force of imbibition by the protoplasm may be the major factor in activating the transpiration stream is indicated by an observation recently made upon some shrubbery growing on the Stanford campus. Just before the first week in July, 1931, a mass planting of *Choisya ternata* and another of *Coprosma baueri* were pruned down to a uniform height, exposing the shade leaves. This was followed by a period of record-breaking, hot, dry weather (fig. 4). The shade leaves were desiccated, while other leaves upon the same plant, but previously exposed to the sun, were uninjured. Since SPONSLER (**98, 99**), by X-ray crystal analysis methods, has shown a remarkable uniformity to the molecular structure of cellulose, it is difficult to conceive of any difference in the submicroscopic

pores of the cell walls; and the tension exerted upon the water from evaporation in these pores would be the same in both cases. In the case of the shade leaves, this tension was not sufficiently transmitted through the protoplasm as imbibition pressure to provide the tissues with adequate water. In the hardier sun leaves, the protoplasm was able by imbibition to obtain and keep suffieient water to prevent desiccation.

No adequate correlation has been found (MILLER **69**) which could serve as an explanation between the rate of transpiration and the morphological differences between the sun and the shade leaves. This does not necessarily mean that more water was evaporated from the sun leaves, although ZALENSKII (**111**) has reported such results. OVERTON (**73**), in his experiments with *Cyperus,* found an increased rate of evaporation from leaves which had been killed. However, this is merely the process of desiccation rather than of transpiration, considered as a part of sap hydraulics; for OVERTON goes on to say, "the percentage of water contained in the plant is very much below that in plants which have not been killed." PEIRCE (**74**) observed a similar phenomenon in leaves injured by sulphur dioxide.

SHREVE (**94**) found that "transpiring power" was inversely proportional to the capacity of leaves to imbibe water. On this basis there would be less transpiration from the sun leaves than from the shade leaves, which was indicated by the observations on *Choisya* and *Coprosma* just given. These opposite points of view are reconciled by the work of KISSELEW (**50**), who found that more water is transpired from sun leaves than shade leaves; but that in the case of *Syringa vulgaris* and *Caragana arborescens,* under critical conditions, the sun leaves were better able to resist the transpirational loss than were the shade leaves.

Since any osmotic tensions must be transmitted through the protoplasm, the imbibition power of the protoplasm becomes the determining factor in the activation of sap hydraulics.

8. Application of tensile fluid-imbibition theory

MACDOUGAL *et al.* (**65, 66**) have found a very marked zonation in the conducting area in the xylem of each annual ring for several different trees. "In the pine the stream moves through practically all parts of each annual ring; in the willow it moves through the late summer wood only of each annual layer; in the alder through the early spring wood only; in the walnut it moves through the early spring wood and late summer wood of such annual layers as have not been transformed into heart-wood."

When first reported, this zonation was attributed to such anatomical features as perforations in the pit membranes, tyloses, and apical connections with the transpiratory systems in the leaves. In the latter paper it is "ascribed to gases in definite portions of each annual layer." No explana-

tion is offered to account for this latter phenomenon. My earlier study offered in explanation the hypothesis that "the transpiration stream flows in the largest tracheae in which a tensile column can be maintained in the environment of the moment."

That the rate of flow is not the same in all vessels is readily observable through the horizontal microscope, using a translucent stem and a dye such as eosin. Flow will take place most readily through the larger vessels, since there is less friction in them. This can also be observed through the microscope by sectioning a branch, which has been transpiring in eosin solution, at the point of farthest advance of the eosin. At the same time flow is taking place more slowly in the smaller vessels.

Moreover, the smaller the vessel the greater the proportion of wet wall to maintain the sap under conditions of tension. Therefore, bubbles of gas will form in the larger vessels under excess transpirational pull before they will in the smaller. In a sufficiently small vessel, the influence of sorption may extend sufficiently near to the center of the vessel to prevent the formation of gas bubbles.

That these theoretical premises are correct is shown by the work of BODE (**12**), who observed, by careful dissection and direct observation, that air entered the largest vessels first. "Dagegen war das Eindringen von Luft in Gefässe mit einem Durchmesser unter 10 μ nie zu Beobachten."

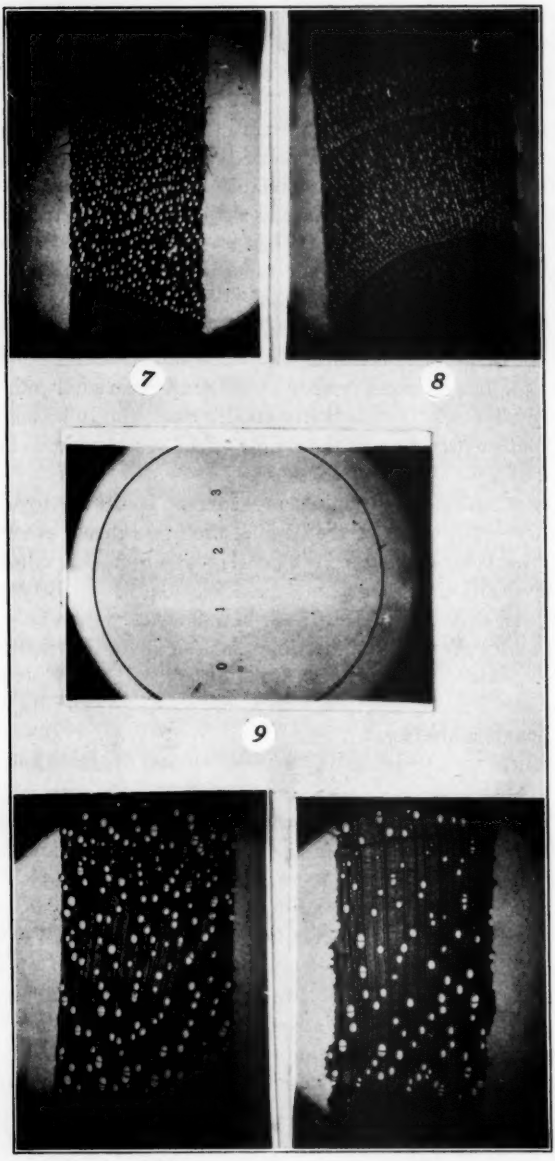
Through the courtesy of Dr. D. T. MACDOUGAL, I was able to examine the original slides prepared at the time that the above-mentioned studies on zonation were made. Photomicrographs of typical annual rings are presented in figures 7 to 11, together with a photomicrograph of a stage micrometer to the same scale (fig. 9).

The exactness with which the hypothesis offered fits these cases is further evidence in its favor.

In the pine (no illustration), where there are only tracheids and no tracheae, all of the elements are fairly uniform in diameter, with cross walls at frequent intervals; therefore there is conduction in all of them.

In the willow (fig. 7), each annual ring immediately starts off in the spring with large vessels, which continue until late summer when the size falls off rapidly and they become more numerous. The line of demarcation is sharper here than in the other two cases given later. According to my hypothesis, under conditions of stress, flow should take place only in the last millimeter (or less) of the annual ring. MACDOUGAL *et al.* report that "under midsummer conditions conduction is only in the later summer wood. Irrespective of the width of the annual ring, the width of the colored zone in the late summer wood is less than a millimeter."

In the alder (fig. 8), there are numerous and comparatively small vessels formed in the spring, becoming more numerous and rounded out as the



FIGS. 7–11.—Fig. 7, photomicrograph of transverse section of an annual ring from the xylem of willow. Fig. 8, same of alder. Figs. 10, 11, same of walnut. Fig. 9, photomicrograph of stage micrometer at same magnification as figures 7 to 11.

season advances, being very few in late summer; therefore the sap flows through the spring wood under midsummer conditions; my hypothesis again correlating with the observed fact.

In the walnut (figs. 10, 11), it was found that "the dye moves through the outer portion of the late summer wood and the innermost portion of early spring wood (of untylosed annual rings), leaving an intermediate zone through which there is no conduction. The zones of coloration are not as clearly defined as in the willow and alder." Applying my hypothesis, the reason for this is apparent on looking at the relative sizes of the vessels throughout the ring, small ones being formed on both edges with less distinct lines of demarcation than in the other two cases. Two rings chosen at random were photographed in this case to show the general applicability of the theory.

This hypothesis can be applied in ecological fields. For example, those plants with large ducts adapted to transporting water under moist conditions with mild transpiration, such as *Echinocystis,* succumb early under adverse conditions, compared with those plants having smaller vessels. The size of the ducts, to a large extent, is a hereditary factor. I have observed *Montia* growing in very dry and in very moist habitats, both of which had mature vessels 25 μ in diameter, the difference being in the number of such vessels. The hypothesis that the transpiration stream flows in the largest vessels in which an imbibed tensile fluid can be maintained under the conditions of the moment should prove to be a valuable addition to sap hydraulics.

Summary

1. The transpiration stream moves through a system of xylem vessels, the walls of which are wet by the water moving through them. The xylem is surrounded on all sides and terminally by living cells. Flow through the xylem is a problem in hydraulics. The DIXON-ASKENASY tensile column theory is inadequate. Combined with the SACHS-LANGMUIR ideas on imbibition, a satisfactory hypothesis is obtained under which the walls of the vessels imbibe and support the weight of the water moving through them by tensile stress.

2. The corollary is offered that flow takes place through the largest vessels in which an imbibed tensile fluid can be maintained under the conditions of the moment. This can explain the observed zonation of conducting areas in the xylem, since the latter have vessels of varying diameters in definite seasonal zones.

3. The products of photosynthesis are transported through the phloem in solution. This solution is dehydrated when the nutrient materials are incorporated into growing cells, the excess water escaping by the path of

least resistance. A consideration of the morphological relations of phloem, meristematic tissue, and xylem, together with experimental evidence from guttation, shows that this phenomenon can account for root pressure and serve to repair broken water columns in the xylem.

4. In the absence of root pressure, the entry and movement of the transpiration stream can best be explained through tensile forces, arising by insolation in the mesophyll cells of the leaves, and transmitted through the forces of imbibition, osmosis, and cohesion. Evaporation against the imbibition pressure of protoplasm may be the determining factor in the activation of sap hydraulics.

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EFFECT OF SOIL TYPE AND AERATION UPON ROOT SYSTEMS OF CERTAIN AQUATIC PLANTS

B. ELIZABETH DEAN

(WITH SIX FIGURES)

Introduction

It is well known that the amount and movement of soil air are important factors in plant development. Because of the fact that many crop and aquatic plants are often forced to develop under conditions of minimal soil aeration, many investigations have been undertaken to determine the effects of restricted soil oxygen availability (**1, 5, 7, 8, 14, 19, 20, 24**). Such studies have disclosed that aeration is important in root respiration (**8, 12, 20**), nutrient absorption (**5, 6, 18, 20**), rate of elongation (**2, 6, 7, 8, 13, 21, 24**), development of hair roots (**11, 23, 24**), aerenchyma formation (**3, 16, 21**), adventitious root development (**10, 24, 25**), as well as in modifications of the gross anatomy of the entire root system (**6, 8, 9, 24**). The many papers reviewed by CLEMENTS (**8**) indicate that the rôle of oxygen in root activity is a primary factor influencing, and in wet soils controlling, the functions of the root system that may determine the destiny of the plant. But few studies (**2, 4, 17**) have been made of root response in greatly increased soil aeration, although some data are available on the effects of culture solution aeration (**6, 13, 18**).

This report embodies results of an experiment to determine the effects of soil aeration, increased well above the maximum usually encountered in nature, on several species of plants with relatively low oxygen requirements.

Materials and methods

Four species of aquatic seed plants, *Hibiscus militaris, Acorus calamus, Sagittaria latifolia,* and *Typha latifolia,* were selected for use in the investigation. In the selection of all material of a species, as nearly uniform specimens as possible were obtained. All of the plants were placed in tanks in 10 inches of soil, with a 6-inch layer of tap water above the soil surface.

Growth was observed in three types of aerated and unaerated soils: clean coarse sand, highly colloidal clay, and muck. The aeration equipment consisted of perforated, coiled brass pipes at the bottom of each tank, fed through a pipe attached to the air-line with rubber tubing. Frequent titration and H-ion tests were made to check the alkalinity of the cultures. Slight alkalinity was partially maintained by the use of weak alkaline tap water to replace the water evaporated from the tanks. The investigation

was conducted in an experimental greenhouse with light, humidity, and temperature maintained as uniformly as possible. Measurements and photographs were made for comparative study of the entire root systems, together with details of the characteristic roots removed from the plants.

Data and discussion

The results of the root development are given separately for each species, and are followed by observations correlating the root and top growth. Tables I–IV record the data of the investigation, and show the relative degree of root and top development of the four plants in each type of soil.

During the 15 weeks of growth period and at the time the plants were removed and critically studied, *Hibiscus militaris* showed several pronounced modifications. The plant in unaerated sand gave early evidence of shallow root development, for as soon as the above-ground shoot reached a height of a few feet it became top-heavy. After the water was siphoned from the cultures, the roots were found growing on and just under the surface of all unaerated soils. The plants were removed, and without exception the roots in soils of low oxygen content developed a shallow horizontal growth in striking contrast to the vertical growth of the roots through the aerated soils. Small, dwarfed root systems were pronounced in unaerated clay and muck (fig. 1, *A*, *B*). The aerotropic response of root growth to the source of air became evident when air was introduced from the bottom of the soils (fig. 1, *C*, *D*).

The development of heavy clumps of fibrous roots was found in aerated clay and muck. This clumping of roots, not found in sand, was probably influenced by soil nutrition. The root primordia were from the old root stalk in all soils, although adventitious roots developed 10–15 cm. above the root stalk on the plants from aerated substrata. The origin of roots from the old root stalk was supplemented by the development of clumps of fibrous members from the cut ends of old woody roots in all aerated soils and in unaerated sand.

It is apparent from the data (table I) that roots were modified by aeration. Some of the roots became decidedly woody in aerated cultures, but all remained succulent and flexible under unaerated conditions. A comparison of results also showed greater elongation of roots in all aerated cultures than in any unaerated soil. The rate of root and shoot elongation has been increased by aerating the substratum by ALLISON and SHIVE (**2**), HALL, BRENCHLEY, and UNDERWOOD (**13**), and HUNTER and RICH (**17**). Retarded growth and injury in higher plants, according to LIVINGSTON and FREE (**20**), may be attributed to decreased absorbing power of the root as a result of deficient soil oxygen. There was likewise more pronounced elongation of roots in aerated clay and muck cultures than in aerated sand.

TABLE I

ROOT AND TOP DEVELOPMENT IN *Hibiscus militaris* IN DIFFERENT SOIL CULTURES

PLANT DEVELOPMENT	ROOT DIMENSIONS					
	SAND		CLAY		MUCK	
	UNAERATED	AERATED	UNAERATED	AERATED	UNAERATED	AERATED
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
Average length of woody roots	0.0	30–35	0.0	50–55	0.0	52–58
Maximum length of woody roots	0.0	42.0	0.0	60.0	0.0	65.0
Average diameter of woody roots	0.0	0.3	0.0	0.5	0.0	0.5
Average length of succulent roots	10–15	20–25	5–10	25–30	2–6	25–30
Maximum length of succulent roots	25.0	30.0	13.0	35.0	10.0	35.0
Average length of primary laterals	1–4	4–8	2–5	5–10	1–3	7–12
Maximum length of primary laterals	10.0	12.0	10.0	15.0	5.0	15.0
Average length of secondary laterals	0.0	0.2–0.5	0.1–0.2	0.2–1.0	0.1–0.2	0.5–1.0
Maximum length of secondary laterals	0.0	1.0	0.5	1.6	0.2	2.0
	TOPS					
Number of shoots per plant	1	2	3	5	2	6
Weight of tops in grams	120	155	78	370	89	465

Roots in all soils branched, but though secondary laterals were common in roots of all aerated soils, they occurred only occasionally on the surface roots of unaerated clay and muck, and never on the plants in unaerated sand. Twenty singly branched laterals per centimeter was the maximum amount of branching observed in roots in unaerated sand. Frequent branching was confined to the proximal portion of these roots (fig. 2, *A*), and branches became progressively shorter, as well as more irregular in length and distribution, toward the tip. Roots in the sand near the aeration coils showed more frequent branching (30 per cm.) of primary laterals near the tip. These primary laterals formed 5–20 short secondary branches at their

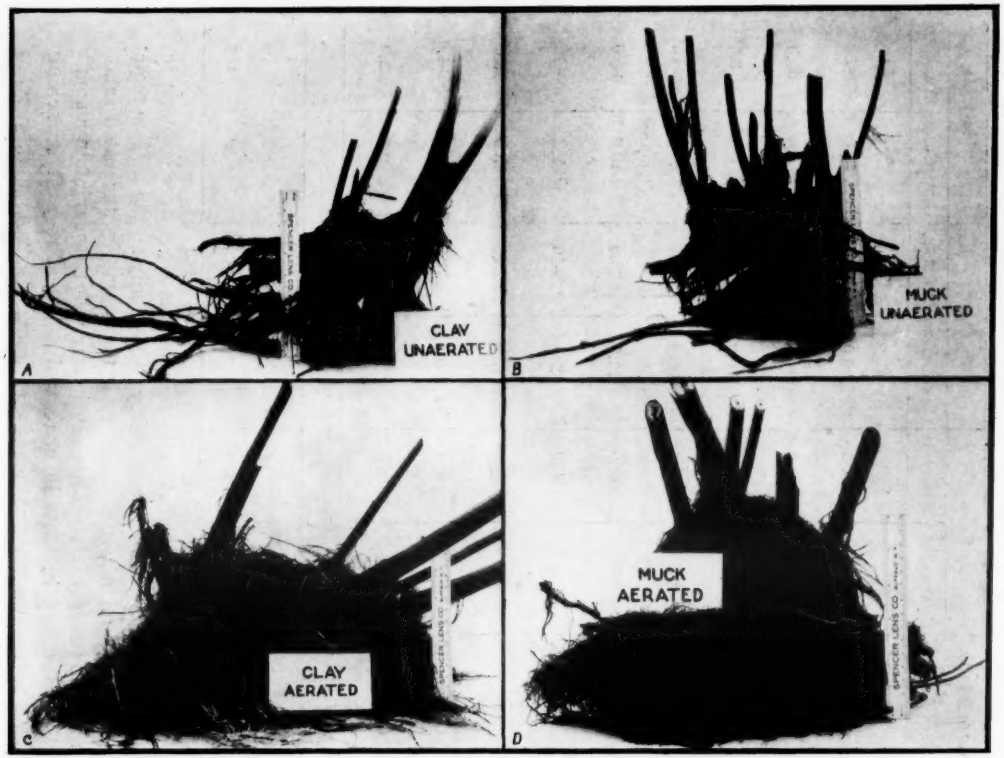

FIG. 1. *Hibiscus militaris* root system: *A*, unaerated clay; stunted root system with short roots developing only from root stalk of original planting. *B*, unaerated muck; meager root system with short fibrous roots developing from upper part of old root stalk. *C*, aerated clay; new woody and fibrous roots ramified throughout entire soil culture and about aeration coils. *D*, aerated muck; extensive system of new woody roots and heavy clumps of fibrous roots; large clump of roots in foreground.

proximal ends (fig. 2, *D*), another character not found on roots from unaerated sand.

Branching of laterals may be observed in roots of both the unaerated and aerated clay and muck cultures (fig. 2, *B, E, C, F*). These root details show that lateral branches are longer and more numerous on the roots receiving additional aeration (fig. 2, *D, E, F*). The irregular branching of laterals of first and second rank on roots from unaerated cultures is also in striking contrast to the long, evenly distributed laterals of respective rank on the roots taken from a clump near the air-lines. Lateral branching is restricted to the upper proximal end of roots from unaerated soils, but roots supplied with aeration have laterals of first and second rank practically the whole length of the roots.

Surface roots in unaerated clay and sand did not possess root hairs on branches that projected into the water, but they were found sparingly on the same type of roots in the muck cultures. Root hairs were observed on all roots of aerated cultures, but most abundantly on roots from the muck and clay. The most outstanding effects of aeration on *Hibiscus militaris*, particularly in compact clay and muck soils, were the marked increase in depth and extent of root growth, with the formation of secondary laterals in all soils.

The root systems of *Typha latifolia* in the different soils and aeration were examined after 56 days' growth, and again 44 days following replanting. All of the types of roots reported by WEAVER and HIMMEL (**24**) and by ROERDON (**21**) in their experiments with *Typha latifolia* were observed. These included: (1) branched water roots found abundantly in unaerated soils that developed at the highest point of origin on the basal node and grew upward and outward into the water to branch on or above the soil surface; (2) branched surface soil roots found in all unaerated soils and aerated sand; (3) deep vertically growing branched soil roots characteristic of all aerated soils; (4) unbranched soil roots found in all cultures. The results following alteration of soil or air factors illustrate clearly the facility with which this plant adjusts its type of root development to its physical environment.

The data (table II) show that branched water roots are found only in the unaerated cultures, and are noticeably longer and more branched in the muck soil than in either of the other soils. The water roots originated at the highest point of the basal node and grew up and out upon the soil surface. Approximately one-third of the roots on the plants in the unaerated cultures were water roots. These roots correspond to the superficial adventitious roots observed by EMERSON (**10**) on *Alnus* and other land plants growing in swamp land, and by WEAVER and HIMMEL (**24**) on *Typha latifolia* in their water-saturated cultures.

heavy clumps of fibrous roots; large clump of roots in foreground.

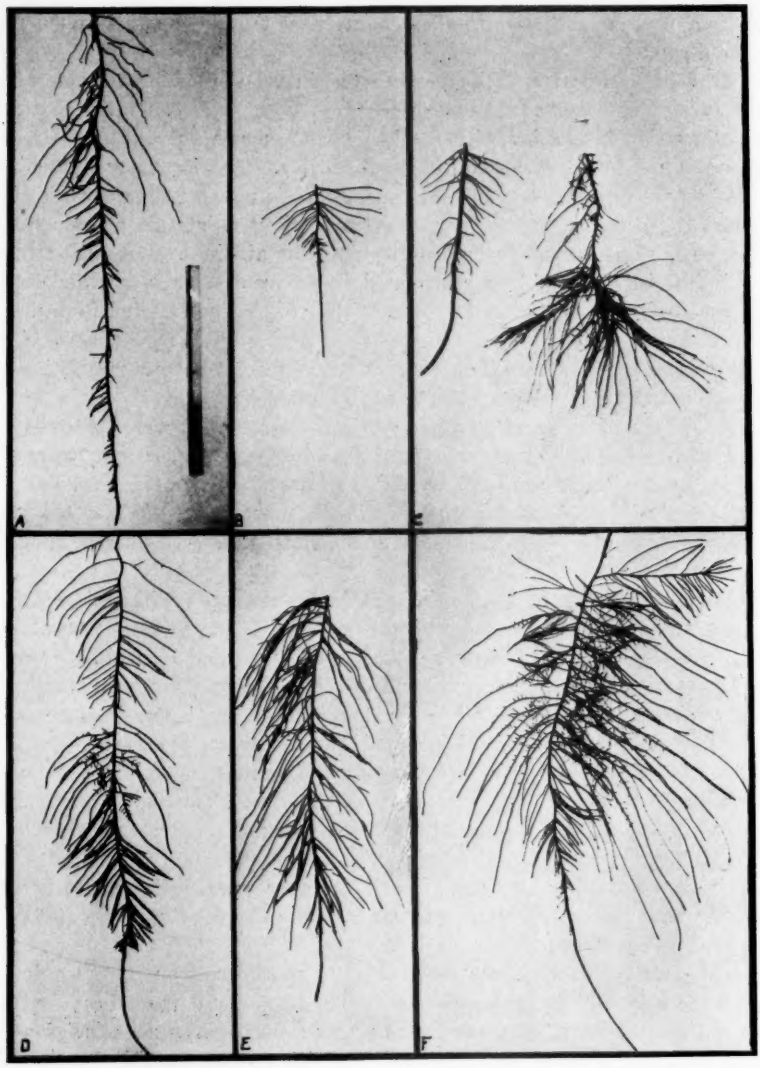

FIG. 2. Details of *Hibiscus militaris* soil roots: *A*, unaerated sand; typical root with primary laterals formed most frequently at proximal portion of root. *B*, unaerated clay; root showing a few short secondary laterals. *C*, unaerated muck; short scattered secondary laterals appearing at proximal region of roots. *D*, aerated sand; primary laterals showing secondary branching. *E*, aerated clay; typical root with secondary branching taken from near aeration coil. *F*, aerated muck; root with long primary and secondary laterals taken from clump of roots in region of aeration coil.

TABLE II

ROOT AND TOP DEVELOPMENT IN *Typha latifolia* IN DIFFERENT SOIL CULTURES

PLANT DEVELOPMENT	ROOT DIMENSIONS					
	SAND		CLAY		MUCK	
	UNAERATED	AERATED	UNAERATED	AERATED	UNAERATED	AERATED
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
A. Branched water roots						
Average length	12–18	0.0	15–20	0.0	20–25	0.0
Maximum length	22.0	0.0	25.0	0.0	32.0	0.0
Average diameter of roots	0.08	0.0	0.1	0.0	0.1	0.0
Average length of primary laterals	2.0	0.0	1.5	0.0	2.5	0.0
B. Branched soil roots						
Average length	15–20	28–35	15–20	35–40	20–25	40–45
Maximum length	30.0	46.0	28.0	50.5	30.0	54.0
Average diameter of roots	0.1	0.15	0.15	0.2	0.15	0.25
Average length of primary laterals	1–2	4–5	0.5–1	4–5	0.8–1.2	5–7
Maximum length of primary laterals	2.5	6.0	1.0	7.5	1.8	10.0
Average length of secondary laterals	0.0	0.2	0.0	0.2	0.0	0.3
C. Unbranched roots						
Average length	10–15	20–25	20–25	35–40	35–40	40–45
Maximum length	24.0	40.0	47.0	56.0	50.0	68.0
Average diameter of roots	0.15	0.15	0.18	0.2	0.2	0.2
D. Rhizomes						
Average length	20–25	25–30	30–35	50–60	65–70	80–85
Average diameter	1.0	1.0	1.0	1.2	1.2	1.5
	TOPS					
Average length of leaves in centimeters	90	100	100	125	110	156
Number of leaves in sheath	5–7	5–7	5–9	7–10	7–12	9–12
New aerial shoots on a rhizome	2	5	4	9	20	39

In unaerated sand, 20–30 laterals per cm. developed uniformly to near the root tip. The water roots from unaerated clay and muck had from 30–50 branches per centimeter, and table II shows that they were 5–10 cm. longer than water roots from the sand soil. The branching was most irregular but proceeded to near the tip on roots from the unaerated muck.

Unaerated and branched roots of lower origin than the water roots grew into the upper 5–10 cm. of the unaerated soils. Unbranched and branched roots in aerated soils grew downward to the region of air supply, with only occasional roots growing horizontally near the soil surface. The longest roots and the most profuse branching of laterals were in the region of the aerating pipes, while branching in unaerated conditions was on the upper half of each root. Secondary laterals never occurred on branched roots of unaerated soil, but were found on roots provided with air.

Branched and unbranched soil roots were consistently longer in the aerated soils. The branched roots in both sand cultures were slender, with wrinkled surfaces. Laterals were correspondingly longer and twice branched in aerated soils (fig. 3, *D, E, F*). Singly branched laterals of roots from unaerated soils were confined to the upper half of the root (fig. 3, *A, B, C*) while the twice branched laterals of roots from aerated soils developed down to the region of root elongation. Several centimeters of the distal ends of roots from aerated cultures were broken off as the roots were removed from the aeration coils. The average of several counts showed that the frequency of primary laterals on the soil roots was 5–30 per cm. from unaerated sand, and 20–40 per cm. from aerated sand. In unaerated clay, single branches developed 20–30 per cm., and in aerated clay 50–60 per cm. The most frequent branching was found on roots from the muck soils, 45–60 per cm. in the unaerated condition and 50–65 per cm. with improved aeration. Lateral branching of soil roots in the aerated muck soil of this experiment showed a considerable increase in frequency over the lateral branching of similar roots reported by WEAVER and HIMMEL in their best aerated moist culture.

The unbranched roots were in the minority in all unaerated soils, but were about equal in number to the branched soil roots in aerated cultures. From the data given in (C) of table II, it is apparent that unbranched roots are more extensive and more abundant in the muck soils than in clay or sand.

Root hairs were found only occasionally on water roots in unaerated muck. While they occurred on all soil roots, they were most numerous on roots from aerated muck and clay. Root-hair development was influenced by aeration, soil nutrition, and position of roots. The fact that they were practically lacking on surface-growing roots in unaerated soils is in accordance with observations of WEAVER and HIMMEL. Their occurrence on water roots of *Typha latifolia* and surface-growing roots of other plants in aerated muck may be explained, according to FARR (**11**), by the fact that calcium was present in the muck soils. SNOW (**23**) also, found that an increase of available oxygen produced root-hair development on various plants.

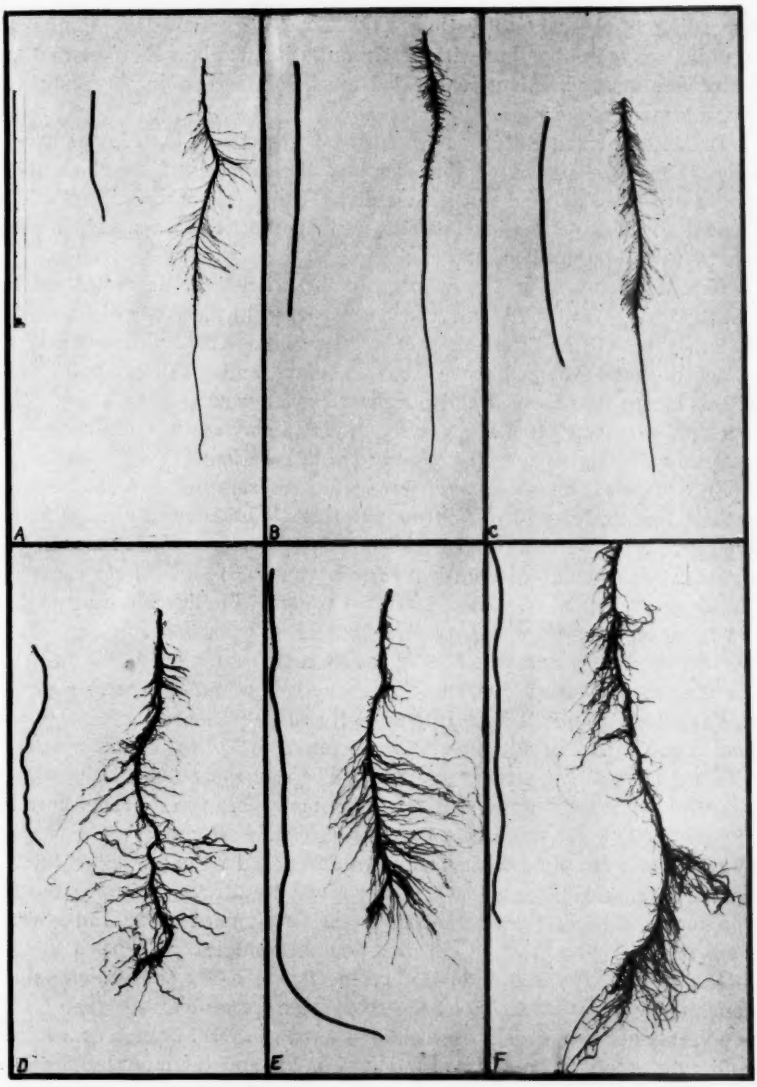

FIG. 3. Details of *Typha latifolia* soil roots. *A*, unaerated sand: (*a*) unbranched root, (*b*) branched root with single laterals on upper half. *B*, unaerated clay: (*a*) unbranched root, (*b*) branched root with single laterals confined to proximal region. *C*, unaerated muck; (*a*) unbranched root, (*b*) branched root with single laterals. *D*, aerated sand: (*a*) kinky unbranched root, (*b*) branched root with occasional laterals of second rank. *E*, aerated clay: (*a*) unbranched root, (*b*) branched root with secondary laterals. Distal end broken in removing from aeration coil. *F*, aerated muck: (*a*) unbranched root, (*b*) branched root with secondary laterals. Root tip broken off.

According to these investigations (**11, 23**), the presence of calcium and abundant oxygen, together with better nutrition, explain the fact that root hairs were most abundant in aerated muck soil, second in clay, and least in sand.

In the unaerated cultures new rhizomes were fewer than in the aerated soils. The development of rhizomes and the number of aerial shoots on plants grown in aerated muck exceeded those in all other cultures. One aerated specimen of *Typha latifolia* developed 12 rhizomes and 39 aerial shoots, with two rhizomes 125 cm. long.

Root development of *Typha latifolia* varied with the degree of aeration and the type of soil. These variations occurred in the mode of branching, the general habit, and the extent of development. In oxygen-deficient soil, branched roots developed above the soil surface, and with abundant oxygen all roots grew into the soil and downward to the source of aeration. Root development was greater in muck soils than in clay and sand, although the growth in all soils was greatly increased with aeration.

Specimens of *Acorus calamus* were removed from the cultures for study of their root systems 70 days after planting. Following the examination, they were replanted and taken up again at the end of 40 days. The root systems were made up of branched and unbranched roots, the former occurring in greater number. A comparative study of the development of these two types of roots showed a greater number of branched roots in aerated sand but a greater number of unbranched roots in aerated muck and clay. The branched roots were strong though very fine and hairlike.

Variations in growth with respect to the soil were more comparable with those found in *Hibiscus* than with those found in *Typha*, as differentiation of branched roots was not pronounced. The root systems in the unaerated soils (fig. 4, *A, B*) were shallow, with many branching roots forming tangled mats, while those in aerated soils (fig. 4, *D, E*) grew downward toward the air supply. Branched and unbranched roots as well as laterals were, without exception, longer in the aerated cultures. Neither the soil type nor aeration had any influence on the development of second laterals, as the branching habit in all cultures remained single.

A comparison of the details of roots (fig. 5, *A–F*) shows that lateral branching was less extensive on the roots from unaerated soils than on the deep vertical-growing roots from aerated soils. Lateral branching was outstanding on roots from aerated sand (fig. 5, *D*), and the laterals were 5–10 cm. longer than in roots from unaerated sand. Measurements (table III) show that laterals were fewer and 2–3 cm. shorter on roots from unaerated muck than from unaerated clay. Improved aeration produced a marked effect upon the growth of laterals in these two soils, the length being four times greater in aerated than in unaerated muck.

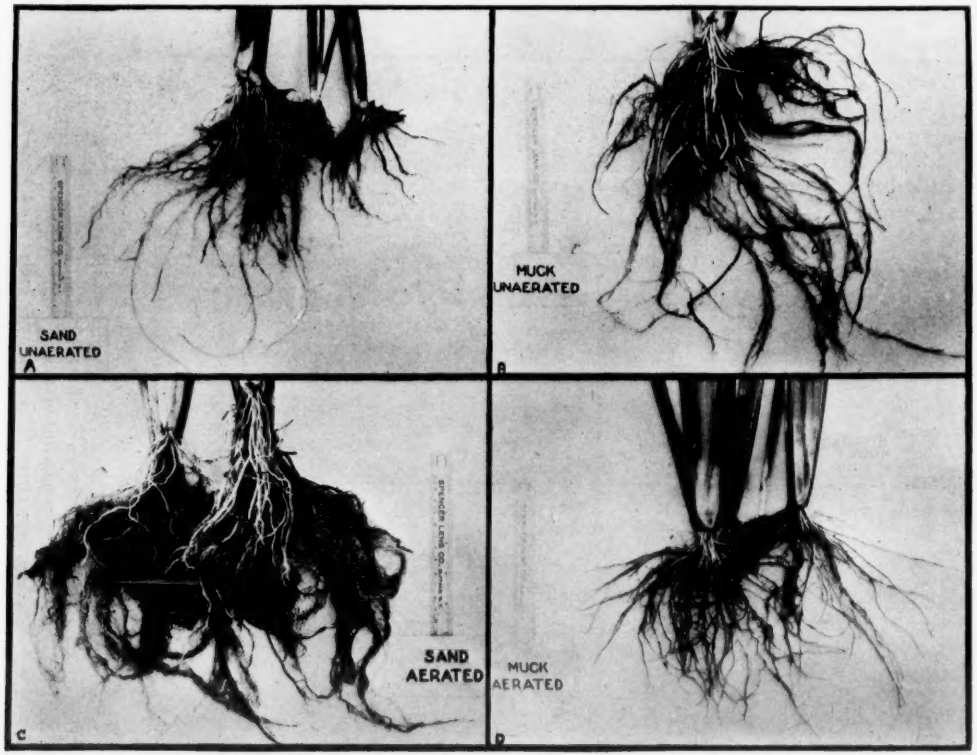

FIG. 4. *Acorus calamus* root system: *A*, unaerated sand; wrinkled roots with short laterals growing horizontally under sand. *B*, unaerated muck; roots growing in horizontal position on surface and upper regions of muck. *C*, aerated sand; roots showing kinky configuration from growing into sand. *D*, aerated muck; many roots broken and lost on removal from aeration coils.

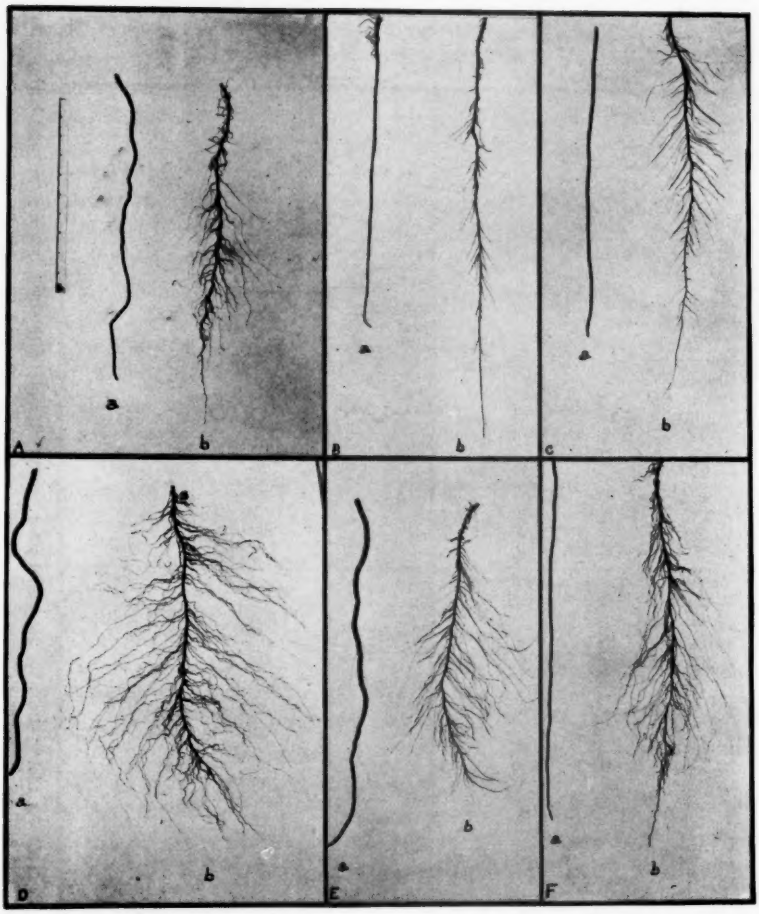

FIG. 5. Details of *Acorus calamus* soil roots. *A*, unaerated sand: (*a*) unbranched root, (*b*) typical branched root from same soil. *B*, unaerated clay: (*a*) practically unbranched root, (*b*) branched root with scattered laterals. *C*, unaerated muck: (*a*) unbranched root, (*b*) branched root from same culture showing shorter branching toward distal end. *D*, aerated sand: (*a*) unbranched root, (*b*) branched root from same plant with long delicate laterals developing down to root tip. *E*, aerated clay: (*a*) unbranched root, (*b*) branched root with lateral branching to tip. *F*, aerated muck: (*a*) unbranched root, (*b*) branched root with laterals developing to zone of elongation.

TABLE III

ROOT AND TOP DEVELOPMENT IN *Acorus calamus* IN DIFFERENT SOIL CULTURES

PLANT DEVELOPMENT	ROOT DIMENSIONS					
	SAND		CLAY		MUCK	
	UNAERATED	AERATED	UNAERATED	AERATED	UNAERATED	AERATED
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
A. Branched root system						
Average length	13–20	23–30	18–22	30–35	20–25	30–35
Maximum length	25.0	35.0	30.0	40.0	30.0	40.0
Average diameter at proximal end	0.12	0.15	0.12	0.15	0.13	0.12
Average length	1–2	8–12	1–2	5–7	0.5–1.5	7–10
Maximum length of primary laterals	6.0	15.0	6.0	10.0	3.0	12.0
B. Unbranched root system						
Average length	7–12	15–20	18–22	20–25	20–25	25–30
Maximum length	20.0	30.0	32.0	40.0	35.0	40.0
Average diameter	0.12	0.15	0.15	0.2	0.2	0.18
	TOPS					
Average height in centimeters	400	450	420	500	450	500

Root hairs were not observed on the surface roots of *Acorus calamus* in any of the oxygen-deficient soils, but occurred on the roots from aerated muck and clay.

The root habits just described show the same aerotropic response as *Hibiscus militaris* and *Typha latifolia*. The influence of the soil or aeration, however, was not observed to induce an increase of additional ranks of laterals as was found to occur with the *Hibiscus* and *Typha*.

Sagittaria latifolia plants were examined 44 days after planting. Branched roots developed at the base of the leaf stalks around the crown of the old rhizome which was used in the original planting. Only the very young roots were unbranched, a root response not following in the other species. All roots were singly branched and intricately tangled. In no case was lateral spreading of roots found on the soil surface, but all unaerated systems presented shallow horizontal growth away from the points of origin. Their branches were irregular and extremely stunted in comparison with those where aeration was supplied (fig. 6, *A*, *B*). The roots in the aerated soils grew to the air-lines, where they formed an interlaced

mesh of laterals throughout their extent of growth, and were frequently broken when removed from the aerating system.

Much bulkier root systems developed in unaerated sand than in clay and muck cultures with similar aeration. *Hibiscus militaris* showed like response to the same soil conditions. In contrast, *Typha latifolia* and *Acorus calamus* produced greatest growth in muck soil without aeration. *Sagittaria* likewise developed the greatest number of, and the most uniformly branched, roots in the sand of the aerated cultures. Although no nutrients other than those in the tap water were added to the sand, these cultures rather than clay and muck favored the growth of *Sagittaria*.

TABLE IV

ROOT AND TOP DEVELOPMENT IN *Sagittaria latifolia* IN DIFFERENT SOIL CULTURES

PLANT DEVELOPMENT	ROOT DIMENSIONS					
	SAND		CLAY		MUCK	
	UNAERATED	AERATED	UNAERATED	AERATED	UNAERATED	AERATED
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
Average length of roots	5–10	30–35	4–8	20–25	3–5	22–25
Maximum length of roots	15.0	42.0	12.0	35.0	10.0	35.0
Average length of primary laterals	0.5–1	3–5	0.2–0.5	1–3	0.5–1	1–5
Maximum length of primary laterals	1.5	6.0	1.5	5.0	1.5	2.0
	TOPS					
Number of leaves per plant	5	5	5	5	4	5
Average length of petiole	42	45	32	56	30	39
Average length of blade	13	16	13	23	9	15
Average width of blade	7	10	7	17	6	9

Root elongation was increased approximately three times in this species by improving soil oxygen content of all soils (table IV). Length of laterals was increased four to six times in the aerated sand and clay. The irregularity of branching on the upper half of roots developed in insufficient soil oxygen (fig. 6, *C a, D a, E a*) is in contrast to the regular branching extending down to the region of elongation on aerated roots (fig. 6, *C b, D b, E b*).

Root hairs were found on roots of the *Sagittaria* plants in all cultures, but were more numerous on roots from the sand cultures than had been observed on the other species in similar cultures.

The species selected for this investigation are all hydrophiles adapted to life in poorly aerated soils. Their marsh bog habitats usually comprise a composite soil rich in organic matter. Experimental results (**12, 15, 22**) make it practically certain that injury from the toxic effect of carbon dioxide is more frequent than commonly supposed in all oxygen-deficient soils. From analysis of soil atmosphere, RUSSELL and APPLEYARD (**22**) found that rapid nitrification in organic soils was followed by an increase in carbon dioxide content and a falling off in the amount of oxygen. The percentage of carbon dioxide became extremely high and that of oxygen low in the water-logged soils. The muck cultures comparable with bog soil would have a high carbon dioxide content from decomposition of the organic matter in addition to that resulting from root respiration.

Varied types of soil were used to determine whether aeration produced uniform effect throughout the investigation. It has been pointed out in the results that root habits of all species varied by altering aeration and soil nutrition. *Typha latifolia* (table II) displayed the greatest adaptation to growth in stagnant soils by the development of finely branched water roots. This is evidently a response for securing oxygen peculiar to this plant, as water roots were rarely observed in aerated cultures. The occurrence of water roots probably accounts for the fact that *T. latifolia* plants grew in unaerated clay and muck. Although not so rank as in the corresponding aerated soils, they were by no means so dwarfed as the plants of *Hibiscus militaris* and *Sagittaria latifolia* from unaerated cultures which did not develop water roots.

In considering top development of the plants, it was observed that leaves or aerial shoots appeared from 7 to 12 days earlier in all species planted in the aerated soils. Color differences were not noticed in the foliage of *Sagittaria latifolia* and *Acorus calamus,* but the leaves of *Typha latifolia* and *Hibiscus militaris* grown in the sand cultures and unaerated clay were from the first a lighter green than the same species in the other cultures.

The data (tables I, III, IV) relating to the growth of plants above the soil indicate more abundant top development in all aerated soils for *Hibiscus militaris, Acorus calamus,* and *Sagittaria latifolia. Typha latifolia,* however, had greater growth in unaerated muck than in aerated clay and sand. The most rank top growth for *H. militaris* and *T. latifolia* was in aerated muck, but the tops of *A. calamus* and *S. latifolia* were greater in aerated sand than in aerated muck.

The correlation between root and top development is obvious regardless of the varied effects which were produced upon the species by the soils and

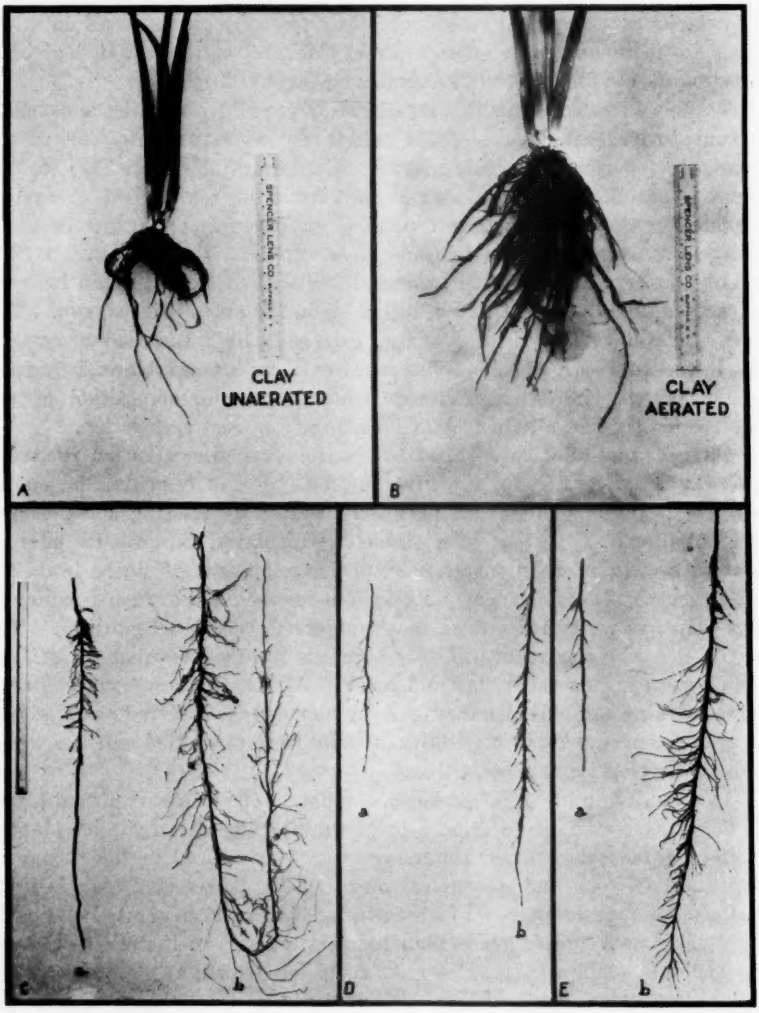

FIG. 6. *Sagittaria latifolia* root systems and root details. *A*, unaerated clay: entire root system stunted. *B*, aerated clay: root system shows effects of aeration. Root ends broken off in removal from aeration coils. *C*, sand cultures: (*a*) typical root from unaerated sand showing development of branches confined to upper half of root, (*b*) root from aerated culture with regular branching to region of elongation. *D*, clay cultures: (*a*) typical root from unaerated culture showing few laterals developed in comparison with (*b*), root from aerated clay showing more regular development of laterals to region of elongation. *E*, muck cultures: (*a*) root from unaerated culture, (*b*) root from soil receiving aeration.

aeration. With increased aeration, there followed uniformly greater top growth than occurred in corresponding plants in unaerated soils. HUNTER and RICH (**17**) found that the rate of transpiration and the intensity of respiration were increased in the shoots of *Impatiens balsamina* with soil aeration. Accompanying these observations there was an increase in surface area of both stem and leaves with increased functional activities.

Hibiscus militaris was the only species to mature. The facts that the plants in aerated cultures flowered normally and that all buds blasted and dropped from the plants in unaerated soils are in accordance with the findings of ALBERT and ARMSTRONG (**1**), who report an increased shedding of cotton fruit buds from plants associated with soil air containing high carbon dioxide and low oxygen percentages. ALLISON and SHIVE (**2**) likewise found that the average total yield of soy beans, when a maximum supply of oxygen was maintained, was greater than yielded from unaerated cultures.

The effects of increased aeration varied with the different species in the different soil types. The results indicate that root and shoot development was increased in all aerated cultures regardless of soil type, while the extent of the plant growth reached a maximum in aerated muck soil, with the exception of *Sagittaria latifolia* which grew best in aerated sand. The deviation from the general behavior, noted in *Sagittaria*, appears attributable to the fact that it possesses a high degree of vegetative activity and ability to develop adventitious roots for aeration. This plant seems to rely more upon its aqueous substratum than upon the soil for its nutrients, especially under conditions of poor aeration.

Summary

1. Soil aeration, involving access to oxygen and removal of carbon dioxide, appears to play an important rôle in plant growth through its direct effect on root systems. Somewhat abundant soil aeration does not appear superoptimal, although it induces structural characteristics not found in plants under approximately normal soil conditions. Although the magnitude of these formative changes in plants varies with the soil type, the general tendency in the species studied was rather uniform.

2. *Hibiscus militaris, Typha latifolia, Acorus calamus,* and *Sagittaria latifolia* developed bulkier root systems and larger tops in aerated cultures.

3. Relative growth and development of all species were greatest in muck, second in clay, and least in sand with the following exceptions: *H. militaris* had a maximum growth in unaerated sand, and *S. latifolia* attained maximum growth in unaerated and aerated sand cultures.

4. The root systems of all species showed aerotropic response. In unaerated cultures the plants established shallow and surface root systems,

while with aeration the roots ramified the soil with the greatest growth in the region of the aerating coils.

5. Increased root elongation was accompanied by corresponding increment in root diameters in aerated soils, with this exception: root diameters of *Acorus calamus* were greater in unaerated muck than in aerated.

6. Laterals were longer and branched nearer the root tips in all aerated soils than in unaerated.

7. Second-rank laterals occurred in *H. militaris* roots in all cultures except unaerated sand, and on *T. latifolia* roots in all aerated soils but never in unaerated ones.

8. Roots of *S. latifolia* and *A. calamus* remained singly branched in all cultures.

9. Branched surface-growing roots were developed by *H. militaris*, *T. latifolia*, and *A. calamus* in all unaerated soils.

10. Root hairs did not occur on surface-growing roots in unaerated sand or clay, but were found in unaerated muck. They were much more numerous on roots from all aerated soils.

11. Pronounced formative changes may be induced by artificially increased soil aeration, and the effects of rapid renewal of the soil atmosphere are by no means so injurious to the plant as restricted soil aeration is apt to be.

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INFLUENCE OF LEAF DESTRUCTION BY SULPHUR DIOXIDE AND BY CLIPPING ON YIELD OF ALFALFA[1]

GEO. R. HILL, JR. AND M. D. THOMAS

(WITH SIX FIGURES)

Introduction

Exposure to a sufficiently high concentration of sulphur dioxide for a long enough period of time always injures the leaves of alfalfa. Almost invariably the fully grown, highly functional leaves show the injury first, and are more severely injured than the rest. If the concentration is high enough, for example, to produce markings within an hour, after possibly three-fourths of this time has elapsed the leaves become noticeably stiffened, as though the cells had greatly increased in turgidity. The turgidity seems to increase until certain areas in the leaf (those along the margins and between the veins, where the intercellular air spaces are most abundant) suddenly become flaccid and take on a water-soaked aspect, giving the leaf a mottled appearance. If the fumigation be continued, additional marginal or interveinal areas on these leaves already affected, and corresponding areas on hitherto unaffected leaves, become similarly flaccid.

With a few hours of sunshine after the fumigation, these flaccid areas bleach almost to an ivory color. The areas of each leaf which do not become flaccid remain green. Usually the green areas remaining after a heavy fumigation lie along the midrib and the principal veins of the leaf. They are separated sharply from the bleached areas.

The marked areas of an alfalfa leaflet after any fumigation may vary from one spot the size of a pin-point to several larger ones, which may involve 50 per cent. or more of the area of the leaflet. A given fumigation may destroy more than 50 per cent. of the green tissue of some leaflets and yet leave 50 per cent. of the leaves entirely unmarked. Basal leaves are not often marked, and leaves that have not attained their growth only rarely. In all of our experience with alfalfa, a marked stem has never been found. When a majority of the green tissue of a leaflet is destroyed by sulphur dioxide, the leaflet curls and frequently drops from the plant.

This type of injury is called "acute." Occasionally another type of sulphur dioxide injury on alfalfa leaves is encountered, a type that mani-

[1] Contribution from the Department of Agricultural Research of the American Smelting & Refining Company, Salt Lake City, Utah.

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fests itself several days after the fumigation. This is evidenced by a rather rapid disappearance of chlorophyll from parts of an otherwise apparently normal leaf, as if the chloroplasts in certain areas of the leaf, three or four days after the fumigation, had ceased to manufacture that unstable pigment. This type of injury is called "chlorotic" (chronic injury by STOKLASA **8**).

What is the effect upon yield of a fumigation which destroys a portion of the leaf tissue? Does the green tissue that remains after the fumigation continue to function, and if so, at how nearly normal a rate? If there is a decrease in yield following a sulphur dioxide fumigation, is that decrease proportional to the leaf area destroyed?

In this paper the fumigation apparatus and the methods of growing, fumigation, measuring the injury, and harvesting the plants are described; also yield data as affected by sulphur dioxide lesions and by clipping are presented. The influence of various environmental and physiological factors on leaf destruction by sulphur dioxide, and upon the absorption of that gas by plants, will be considered in subsequent papers.

Historical review

There are many papers, mostly foreign, on the relations of industrial wastes, particularly sulphur dioxide and sulphur trioxide, to agricultural production. The problem has long been recognized as one of considerable scientific and economic interest. The European investigations have been summarized by HASELHOFF and LINDAU (**1**) and by STOKLASA (**8**). In this country the principal report of the problem is by the Selby Smelter Commission (**2**), which also contains an extensive bibliography with abstracts of most of the earlier papers. VERPLANCKE (**14**) has discussed the subject in a general way; and in a review of the literature by JOHNSON (**3**), reference has also been made to recent investigations.

The earlier investigators gave descriptions of the lesions produced on many different plants, and called attention to the acute type and to the chronic, or chlorotic type of SO_2 markings. They also suggested an order of magnitude of the exposures to sulphur dioxide which might cause injury to vegetation. The Selby Commission developed a technique for fumigating plants with sulphur dioxide similar to that subsequently improved upon by O'GARA, described later, and carried out an extensive study of the action of SO_2 on barley. The Commission's report for the first time gave quantitative information as to the effect of concentration and duration of fumigation upon leaf destruction. They showed that "severe" lesions might reduce the yield of grain as much as 40 per cent., depending on the stage of growth when the fumigation occurred, and that it was somewhat proportional to the intensity of the fumigation. They found no reduction in yield

when the leaves were injured only slightly or not at all. Similarly JOHNSON (**3**), working on the problem of invisible injury, found no measurable injury to wheat even after very prolonged exposure to sulphur dioxide but at concentrations too low to produce visible markings. So far as the writers are aware, there are no investigations in the literature on the relation of leaf destruction by sulphur dioxide to yield of alfalfa.

In 1914 the American Smelting & Refining Company established a Department of Agricultural Research to study the smelter smoke problem. This Department, under the leadership of the late Dr. P. J. O'GARA, carried out a considerable number of experiments during a period of nine years, the details of which, for the most part, remained unpublished in 1923, when O'GARA became ill and was unable to participate further in these researches. Some of the results of these studies, however, were published in a paper by WELLS (**15**), who discussed the conditions favoring the dispersion of sulphur dioxide from a smelter. SWAIN (**9**) and THUM (**13**) have summarized the smoke litigation in Salt Lake Valley, Utah, giving some details of O'GARA's work, including photographs of the apparatus and of the experimental plots in the field. O'GARA discussed some of his conclusions in 1917 (**4**), and also before the American Institute of Chemical Engineers (**5**). Only an abstract of the latter paper was published. Further, O'GARA circulated in a limited way a list which indicated the relative resistance of a number of plants to sulphur dioxide.

Fumigation apparatus and technique

The plan of procedure developed by O'GARA in his closed-cabinet fumigation experiments has been largely followed in these studies. O'GARA used small field plots 5 feet square, suitably spaced in the field so that they were readily accessible. He fumigated these plots with various synthetic mixtures of sulphur dioxide and air, under a cabinet $180 \times 180 \times 120$ cm., made of celluloid mounted on a wooden framework. The sulphur dioxide was delivered from a bottle through a needle valve to an empirically calibrated capillary flowmeter, which indicated the rate of flow of the gas. An adjustable water trap before the needle valve enabled the operator easily to obtain a controlled and very steady stream of gas, which was sent into a fan delivering about 10,000 liters of air per minute. The gas mixture was then conveyed through a 15-cm. pipe to the center of the top of the fumigation cabinet, where radial baffle plates distributed the stream uniformly downward to all parts of the cabinet. As the cabinet was not gas-tight and had no specific outlet, the displaced air-SO_2 mixture leaked out wherever it could. The concentration of SO_2 in the air in the cabinet was determined intermittently by the starch-iodine method of MARSTON and WELLS, as described by the Selby Commission (**2**). This method consisted

of drawing a definite volume of the SO_2-air mixture into a partially evacuated 20-liter bottle containing a starch-iodine solution of known strength, absorbing the SO_2 by vigorous agitation, and titrating this solution with iodine or sodium thiosulphate to a standard light blue color. The amount of SO_2 thus determined by analysis usually agreed well with the values calculated from the volumes of SO_2 and air as indicated by the SO_2 flowmeter and a pitot tube in the air line. Humidity readings were taken at intervals with the whirling psychrometer, and the condition of the plants was observed after the fumigation.

The writers planned to control the humidity and light in the fumigation cabinet, and to measure continuously the sulphur dioxide in the cabinet and also the amount of it absorbed by the plants. O'GARA's apparatus was therefore modified with these objectives in mind.

The fumigation cabinet was of similar type, consisting of a light steel framework $195 \times 195 \times 150$ cm., covered with celluloid, and made nearly gas-tight. It was mounted on a galvanized iron base provided with a 20-cm. outlet pipe on one side, and a trough on top in which a water seal with the bottom of the cabinet could be made. It was found later that a more satisfactory seal could be made by using a pad of felt 2 cm. thick in the trough, instead of the water. The base was placed around the plot, made level, and its lower edge sealed with soil. When the cabinet was placed on this foundation, anemometers in the intake and outlet pipes of the system indicated that about 80–100 per cent. of the air which entered the cabinet went out by the outlet pipe.

The air was analyzed for sulphur dioxide continuously and automatically by means of a sulphur dioxide autometer, as described later. This machine sampled the air on a 2-minute schedule alternately from a point just below the delivery pipe in the top of the cabinet and from the outlet pipe. The unaccounted-for air was assumed to have escaped at the average concentration of intake and outlet gas. Sulphur dioxide was added to the system with an arrangement similar to that used by O'GARA and already described, except that the apparatus was inclosed and thermostated. The intake of the fan was provided with a shutter so that the volume of air delivered to the cabinet could be varied as desired.

The cabinet was wired so that lamps and reflectors could be mounted above the plants. When the cabinet was covered with canvas, definite light intensities could be maintained, depending on the number and size of the lamps employed. It has not yet been practicable, however, to attain light intensities approaching sunlight. For determining light intensity, the cabinet was provided with three thermocouple light meters. These thermopiles had sixteen junctions and were constructed of 5-cm. pieces of no. 24 nickel-chromium : constantan wire. They were mounted in a wooden box

about 12.8 cm. square and 2.5 cm. thick. One set of junctions was spread out in a row in a section of the box provided with a glass window, the junctions being painted black and the interior of the compartment being painted white. The cold junctions were placed in a dark but ventilated section of the box. This arrangement gave a reading of about 50 millivolts with full sunlight intensity. More recently, the new Weston "photronic" cell shunted across a resistance box has been used with excellent satisfaction. With the proper shunting resistance, the cell and thermopile gave nearly identical readings in the range from about 10 to 100 per cent. of full sunlight intensity. At lower light intensities the accuracy and sensitivity of the thermopile decreased, whereas the photronic cell remained useful, even in dense shade. The cell was also practically instantaneous in its action, and readily indicated differences resulting from the angle of incidence or clouds in the sky.

For making humidity determinations the cabinet was provided with wet and dry bulb thermometers. One pair of thermometers was mounted in the upper part of the cabinet and was provided with a small fan to cool the wick of the wet bulb. Another pair was mounted in the base near the outlet pipe where the movement of the air in the pipe furnished sufficient draft to cool the wet bulb. The relative humidity was maintained at any desired percentage by blowing an atomized spray of water into an intake pipe leading to the fan and also by introducing steam into the same intake pipe. The spray alone was found to be insufficient to maintain the relative humidity on dry summer days above 60 to 70 per cent.; but when this supply of moisture was augmented by steam, the humidity could be maintained between 95 and 100 per cent. Use of the water spray alone ordinarily causes the temperature of the cabinet to fall from 1° to 4° below the outside temperature. While such lowering of the temperature might have a slightly adverse influence on the experiment, it was also advantageous in preventing moisture from condensing on the walls of the cabinet. Silica gel was used to lower the relative humidity.

A continuous, automatic analytical method for determining sulphur dioxide in air was a fundamental requirement for this work. This was partly attained in 1927, when a machine was constructed (**10**) which automatically drew a measured volume of gas through a measured volume of standard starch-iodine solution at a uniform continuous rate and discharged the solution into a bottle every two minutes, ready for titrating the excess of iodine. In 1928 this apparatus was modified (**11**) so as to absorb the gas in a slightly acidulated solution of hydrogen peroxide, which oxidized the SO_2 to sulphuric acid. The acid thus produced made the solution a better conductor of the electric current, and it was possible to follow the absorption with an electrical conductivity recorder, thus obtaining a con-

tinuous record of the concentration of the sulphur dioxide. The apparatus has been further modified (**12**), so that it is now well adapted to determine with great precision the absorption of the gas by plants in a fumigation cabinet at both low and high concentrations.

The alfalfa plants used were as uniform and comparable as it was possible to secure them. Twenty uniform vigorous seedlings of "common" alfalfa were selected for each plot, in which there were four rows with five plants in each row. The plants thus spaced, when mature, produced approximately fifty stems to the plant. When in the early blooming stage, these stems had approximately fifty leaves (150 leaflets) to the stem. The plots were irrigated by the flooding method often enough to keep the soil well supplied with moisture.

A considerable number of fumigations of alfalfa were carried out under diverse conditions, which produced leaf destruction to different extents. The percentage of leaf destruction was estimated for each plant, either by counting the leaves that were marked and measuring the marked area, or by comparing with a set of standard marked plants, the percentage of bleached area of which had been carefully measured. The estimates of the individual plants, made independently by different observers, were seldom found to differ by more than 10–15 per cent., and the average values for the plot were found to agree within 4 per cent.

At harvest each plant was cut and tied separately, and its green weight obtained immediately. The plants were then placed on end in the greenhouse until air-dry, and again weighed individually, samples being taken to determine the oven-dried weight of the material. A number of individual plants from various parts of the room were weighed at intervals during the time the other plants were being weighed, and were found to vary only to a negligible extent. Accordingly the air-dried weights of each group of plants were considered to be comparable among themselves.

In working out the yield data, the yield of a treated plot was compared with the yield of the same plot on preceding and succeeding untreated crops during the same year, thereby eliminating any influence of variation between the different plots. The yield of successive crops of alfalfa decreases rather regularly during the season (**6, 7**). Factors were, therefore, determined for each crop by means of untreated check plots scattered throughout the field. By multiplying the yield of each of the crops from each plot by its corresponding factor, the yields of the different crops were placed on a comparative basis. The factors for the 1929 yields were found to be as follows: first crop 1.00, second crop 1.46, third crop 2.00, fourth crop 2.75; and for 1930: first 1.00, second 1.51, third 1.61, and fourth 1.99. It was then possible to compare the yield for any treated plot with preceding or subsequent yields of the same plot when untreated. The yield of a treated

crop divided by the expected yield of that crop gave the percentage of yield as a result of the treatment. This method of calculation was complicated by the fact that, in case of the very severe treatments, the next crop subsequent to the treatment might show a reduction in yield also. In such case comparison was made on the basis of the preceding crop, or of the second subsequent crop. Not more than two of the four crops on any plot were treated during a season.

Alfalfa yield data

A. O'GARA'S DATA.—O'GARA conducted many experiments in which he sought to determine the effect on yield of sulphur dioxide fumigations of varying degrees of severity. He recorded the extent of leaf destruction, but in qualitative terms only. It is therefore impossible to evaluate the experiments in which appreciable markings were observed, although they apparently agree in order of magnitude with our own. Accordingly this material is not considered further.

O'GARA also carried out two important experiments with the idea of determining the yield as influenced by repeated low concentrations which did not produce markings. He fumigated three pairs of plots simultaneously, with and without sulphur dioxide, 30 minutes daily for 27 days. On the subsequent crop he reversed the treatment on 42 days. He expressed the yield of the crops fumigated with SO_2 as the average percentage of the yield of the check plots for both crops. This method tended to eliminate plot differences. Table I has been compiled from O'GARA's unpublished data. This table indicates that so long as fumigations do not produce more than traces of markings, there is no significant reduction in yield resulting from a considerable number of short fumigations.

B. YIELD OF ALFALFA AS AFFECTED BY ACUTE SULPHUR DIOXIDE MARKINGS.—During the season of 1929, a great number of plots were given single fumigation treatments on chosen crops, in which varying percentages of leaf tissue were destroyed. The results are shown in figure 1, in which the yield is plotted against the percentage of leaf area destroyed. Since the number of fumigated plots is so large, it is not practicable to present the individual yield data in a table. In figure 1 the various crops which were treated are shown by different kinds of points, as indicated in the legend. The equation of a straight line through these points, worked out by the method of least squares, is

$$y = 98.6 - 0.265x \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1a)$$

in which y is the yield of dry matter expressed as percentage of the check, and x is the percentage of the total leaf area destroyed. The number, n, of plots fumigated in this experiment was 80, and the standard deviation, S_y, of the individual plot yield from the line is 5.85 per cent. The somewhat high value of the coefficient of correlation, $r = 0.715 \pm 0.055$, indicates

TABLE I

INFLUENCE OF REPEATED FUMIGATIONS WITH SMALL CONCENTRATIONS OF SULPHUR DIOXIDE ON YIELD OF ALFALFA; O'GARA'S DATA, 1915

DATE	CROP	NO. OF FUMIGATED PLOTS	NO. OF CHECK PLOTS	NO. OF FUMIGATIONS PER PLOT	DURATION OF FUMIGATION	CONCENTRATION OF SO_2			YIELD DRY WEIGHT PER PLANT (PERCENTAGE OF CHECK)				LEAF DESTRUCTION
						MIN.	MAX.	AVE.	CROP	AVE. OF TWO CROPS	STAND. DEVIATION	STAND. DEVIATION OF MEAN	
					min.	*p.p.m.*	*p.p.m.*	*p.p.m.*		%	%	%	
8/3–9/4	2nd	3	3	27	30	0.3	2.0	0.91	100	.....	.....	.....	None
9/8–10/3	3rd	3	3	41	30	0.4	2.3	0.62	100	100	12.0	5.0	None
8/3–9/4	2nd	3	3	27	30	0.9	4.1	1.82	83	.....	.....	.....	Slight markings
9/8–10/30	3rd	3	3	42	30	0.7	3.2	1.21	107	95	20.0	8.0	Slight markings

that considerable confidence may be reposed in equation (1a) as expressing the relation between yield and leaf destruction for a single fumigation. The difference between the origin of this curve at 98.6 per cent. yield and at 100 per cent. is without significance, considering the large value of the standard deviation of the yield from the curve. The equation indicates that a fumigation which destroys all of the fully matured leaf tissue of a plant at any given time could be expected to reduce the yield about 28 per cent., and that the destruction of any given percentage of leaf tissue could be expected to reduce the yield by that same percentage of 28 per cent.

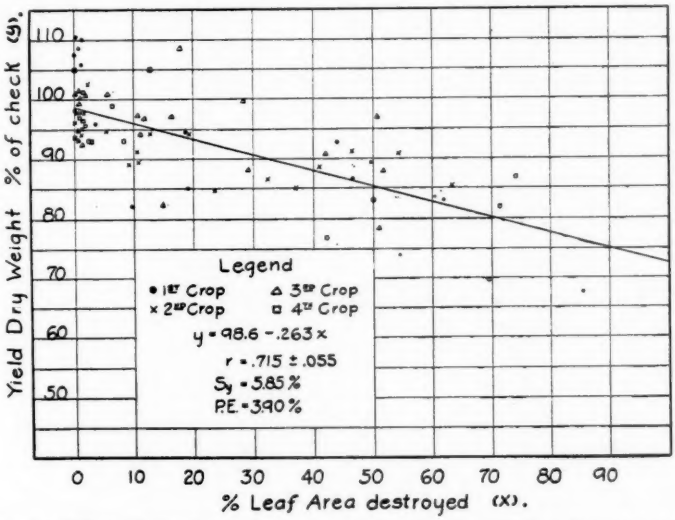

FIG. 1. Effect of the destruction of various percentages of leaf tissue by a single sulphur dioxide fumigation on the relative yield of alfalfa. Each point represents the yield of the fumigated crop from a plot as compared with the untreated crops from the same plot; 1929 data.

In 1930 the attempt was made to produce the same percentage of leaf destruction on certain crops in each of one, two, and three fumigations. The percentage of leaves which were marked was determined, as well as the percentage of the leaf area destroyed. In the multiple fumigations these values are expressed as the average of the individual percentages for each fumigation. These data are presented in figures 2 and 3.

In the upper chart of figure 2 are given the 1930 data. These duplicate the 1929 experiments recorded in figure 1, except that the points are segregated as to stage of growth at time of fumigation, instead of as to the crop fumigated. The equation of the best-fit straight line is:

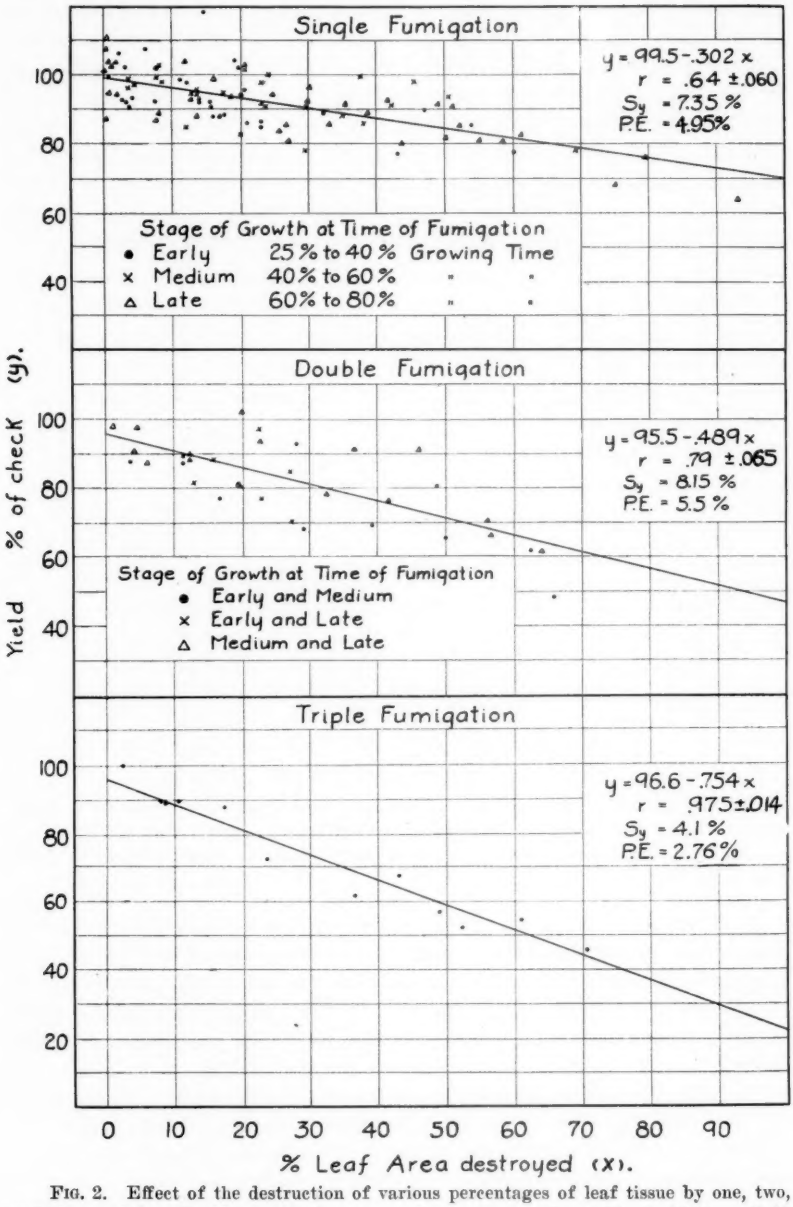
Single Fumigation
y = 99.5 - .302 x
r = .64 ± .060
Sy = 7.35 %
P.E. = 4.95%
Stage of Growth at Time of Fumigation
• Early 25% to 40% Growing Time
x Medium 40% to 60% " "
△ Late 60% to 80% " "
Double Fumigation
y = 95.5 - .489 x
r = .79 ± .065
Sy = 8.15 %
P.E. = 5.5 %
Stage of Growth at Time of Fumigation
• Early and Medium
x Early and Late
△ Medium and Late
Triple Fumigation
y = 96.6 - .754 x
r = .975 ± .014
Sy = 4.1 %
P.E. = 2.76%
Yield % of check (y).
% Leaf Area destroyed (x).

FIG. 2. Effect of the destruction of various percentages of leaf tissue by one, two, and three sulphur dioxide fumigations on a single crop on the yield of alfalfa. Yield of fumigated crop is expressed as percentage of yield of untreated crops on the same plot; 1930 data.

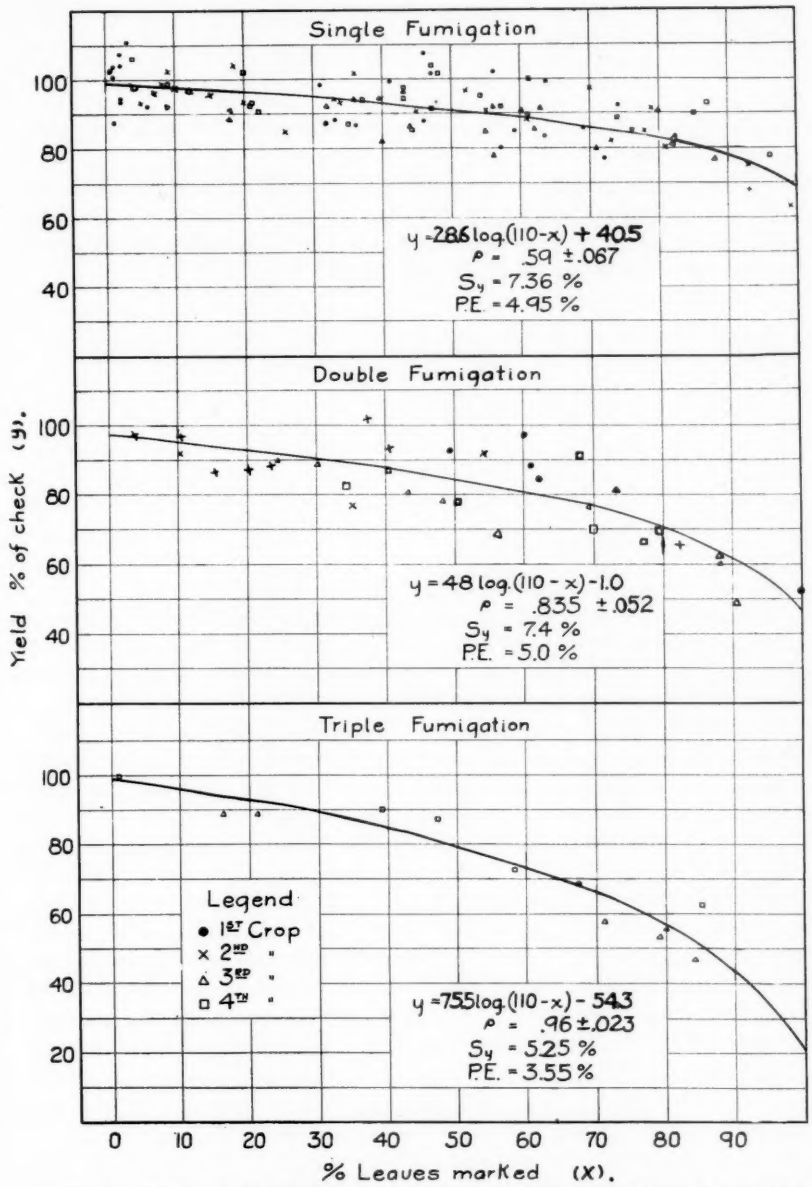

FIG. 3. Effect of the destruction of various percentages of leaf tissue by one, two, and three sulphur dioxide fumigations on a single crop on the yield of alfalfa. Leaf destruction is shown as percentage of the total number of leaves which were marked in any degree. Yield values are the same as in fig. 2; 1930 data.

$$y = 99.5 - 0.302x \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1b)$$
$$n = 96$$
$$S_y = 7.35 \text{ per cent.}$$
$$r = 0.64 \pm 0.060$$

It is apparent that these 1930 data confirm those of 1929 in a very satisfactory manner.

From figures 2 and 3 it is shown that a double or a triple fumigation produces greater reduction of yield than a single fumigation. The equations and statistics of the straight-line relations between the percentage of leaf area destroyed and yield are as follows:

For the double fumigation:

$$y = 95.5 - 0.489x \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$
$$n = 34$$
$$S_y = 8.15 \text{ per cent.}$$
$$r = 0.79 \pm 0.065$$

For the triple fumigation:

$$y = 96.6 - 0.754x \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$
$$n = 12$$
$$S_y = 4.1 \text{ per cent.}$$
$$r = 0.975 \pm 0.014$$

The value of the yield in the case of two complete leaf destructions on one crop is thus about 47 per cent.; and in the case of three complete leaf destructions, about 21 per cent. While neither of these curves strikes the origin at 100 per cent. yield, they both strike within the standard deviation of the values of the yield. The effect of the injury is shown only roughly in both the double and triple fumigation treatments, in spite of the higher values of the coefficient of correlation, r, since the data are hardly sufficient to characterize the curves with a high degree of precision.

In figure 3, which shows the yield as affected by the percentage of leaves which show markings, rather than by the percentage of leaf area destroyed, a straight-line function does *not* fit the data, and a logarithmic equation has therefore been worked out by the method of least squares. The reason for this is that there is a much greater difference between the percentages of leaves marked and the leaf area destroyed with low percentages of the leaf destruction than with high percentages. The equations and statistics of these three curves are as follows:

For the single fumigation:

$$y = 28.6 \log (110 - x) + 40.5 \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$
$$n = 96$$
$$S_y = 7.36 \text{ per cent.}$$
$$r = 0.59 \pm 0.067 = \text{index of correlation}$$

For the double fumigation:

$$y = 48.0 \log (110-x) - 1.0 \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$
$$n = 34$$
$$S_y = 7.4 \text{ per cent.}$$
$$r = 0.835 \pm 0.052$$

For the triple fumigation:

$$y = 75.5 \log (110-x) - 54.3 \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$
$$n = 12$$
$$S_y = 5.25 \text{ per cent.}$$
$$r = 0.96 \pm 0.023$$

In figure 2 the point designations refer to early, medium, and late stages of growth respectively, and in figures 1 and 3 similar designations refer to first, second, third, and fourth crops.

These data indicate that any given percentage of leaf destruction of alfalfa is accompanied by approximately the same percentage of decrease in yield, regardless of the stage of growth or whether it is the first, second, third, or fourth crop, providing time is allowed for the treatment to take effect. Of course a very late fumigation, just before harvest, could hardly be expected to affect the yield; but in these experiments the crop was not harvested until at least seven to ten days after the last fumigation, thus allowing the influence of the fumigation to be fully reflected in the yield. Similarly the alfalfa had been growing a week before the experiments were begun.

The six curves shown in figures 2 and 3 are brought together in figure 4, in order to compare and contrast, in their effect on yield, the different percentages of leaf destruction and the percentages of leaves showing markings. A horizontal line, drawn from any point on any one of the dotted-line curves to the point that it intersects on the corresponding straight-line curve, will indicate the percentage of leaf destruction to which any chosen percentage of leaves showing markings corresponds. Thus, if 50 per cent. of the leaves show markings in one fumigation, approximately 29 per cent. of the leaf area of the plot will have been destroyed, thereby lowering the yield by approximately 9 per cent. Similarly, if 75 per cent. of the leaves show markings, nearly 55 per cent. of the leaf area will have been destroyed, with a corresponding reduction in yield of about 17 per cent. It is interesting to note also that a reduction in yield of 30 per cent. is indicated with a single 100-per cent. leaf destruction, or a double 52-per cent. leaf destruction, or a triple 35-per cent. leaf destruction.

These yield data are based on the average leaf destruction and average yield for plots of about twenty plants. The individual plants in any plot, however, vary greatly at different times, and also vary greatly from one

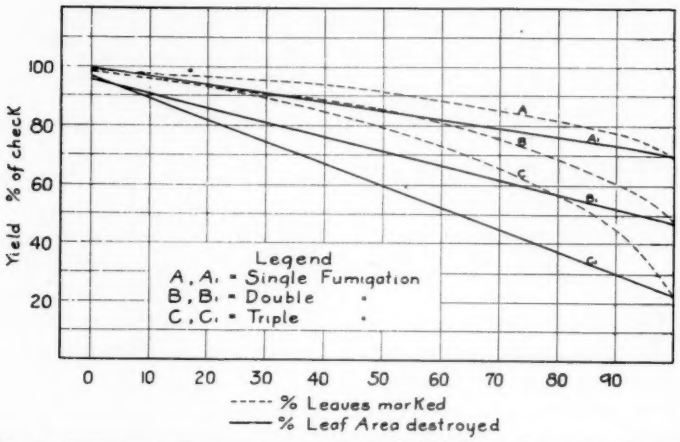

FIG. 4. Summary and comparison of the leaf-yield-destruction curves from figs. 2 and 3. Each curve represents one, two, and three fumigations on a single crop, and the leaf destruction is expressed as percentage of the total leaf area destroyed and as the percentage of the total number of leaves marked to any degree.

another in their ability to absorb sulphur dioxide. While this subject will be treated at length in a subsequent paper, it is desirable to call attention here to the fact of considerable differences between the percentages of markings on the different plants in a plot following a given fumigation. Table II

TABLE II

PERCENTAGE OF LEAF REDUCTION ON NINETEEN DIFFERENT PLANTS IN PLOT 51–2, FUMIGATED AUGUST 2, WITH AN AVERAGE LEAF DESTRUCTION OF 16.2%, AND AGAIN ON AUGUST 11, 1930, WITH AN ADDITIONAL AVERAGE LEAF DESTRUCTION OF 38%

PLOT 51–2, AUGUST 2				PLOT 51–2, AUGUST 11			
Row 1	Row 2	Row 3	Row 4	Row 1	Row 2	Row 3	Row 4
15	30	12	10	30	75	18	60
20	.....	5	8	40	.....	20	25
15	30	25	4	30	55	35	25
50	1	15	12	65	35	15	15
25	2	20	8	65	35	70	10

has been compiled to illustrate these differences. As will be noted in this table, the plants in plot 51–2 were fumigated twice during the same crop, on August 2 when the stems were 16.5 inches tall, and on August 11 when

the same stems averaged 19.8 inches in height. Comparison of the percentages of leaf destruction of different plants in the two fumigations will illustrate the variability in sensitiveness of the individual plants, as well as of the plants in the plot.

It seemed desirable to recalculate some of the yield data with this variability in mind. A number of plants were therefore selected at random from all parts of the field. These plants had all had a single fumigation on one crop in 1930. They were placed in seven groups, each group representing a different percentage of leaf destruction. The yield of each plant was then calculated, using the method already described for the plot yields. Table III presents the average leaf destruction and the average yield of each of these groups of plants, together with the standard deviations of the means of the yield. A comparison of the last two columns of the

TABLE III

RELATIVE YIELDS OF GROUPS OF ONCE-FUMIGATED INDIVIDUAL ALFALFA PLANTS SELECTED AT RANDOM FROM ALL PARTS OF THE FIELD, BASED ON PERCENTAGE OF LEAF DESTRUCTION, COMPARED WITH THOSE CALCULATED FROM EQUATION (1B); 1930 DATA

No. of plants	Leaf destruction		Yields in percentage of check	Yields calculated from equation (1b)
	Range	Average		
	%	%	%	
71	Less than 1%	0.5	104 ± 2.3*	99
68	5–10	8	94 ± 1.6	97
52	20–25	23	94 ± 2.0	93
50	40–45	42	89 ± 2.0	87
29	55–60	57	83 ± 2.6	83
62	70–75	73	81 ± 1.2	78
31	85–95	92	72 ± 2.1	72

* Standard error of the mean.

table indicates the agreement between these yield values and those calculated from equation (1b). Both show very definitely that the decrease in yield of alfalfa is directly proportional to the percentage of leaf destruction.

C. YIELD OF ALFALFA AS INFLUENCED BY CHLOROTIC SULPHUR DIOXIDE MARKINGS.—Thus far in this study, the effects of only the acute type of markings have been considered. The conditions producing the chlorotic type are not very well understood, but they have been observed four or more days subsequent to short fumigations when the light intensity was low and

the concentration of sulphur dioxide was sufficiently high so that under conditions of high light intensity, severe acute markings would have resulted. Chlorotic markings also appear not infrequently after long-continued exposures to low concentrations of sulphur dioxide which are insufficient to produce markings of the acute type. Since chlorosis from a number of widely different causes is common on plants that have not been fumigated, it is frequently uncertain whether, in certain cases, the markings on plants which have been fumigated are due to sulphur dioxide or not.

TABLE IV

RELATIVE YIELD OF ALFALFA AS AFFECTED BY SULPHUR DIOXIDE MARKINGS OF THE CHLOROTIC TYPE

No. of plots	Treatment	Leaf area marked		Yield	Standard deviation	Standard deviation of mean	Yield calculated from equation (1b)
		Acute type	Chlorotic type				
		%	%	%	%	%	%
13	Fumigated	0.5	9.2	96.0	5.35	1.5	96.7

Table IV gives the yield of thirteen plots of alfalfa, upon the leaves of which numerous chlorotic markings were produced. While these data are insufficient to characterize the effect quantitatively, they indicate that the chlorotic lesions are *not more* effective in reducing yield than the same percentage of leaf area destroyed by the acute type of injury.

D. YIELD OF ALFALFA AS AFFECTED BY CLIPPING TREATMENTS.—In 1931, instead of destroying portions of alfalfa leaves with sulphur dioxide, the same range of percentages of leaf reduction as in the 1930 experiments was approximated by clipping off the leaves. These two treatments differed in the fact that, for a given amount of leaf destruction, whole leaves were removed by clipping and normal leaves left, whereas with fumigation, all the leaves were subjected to sulphur dioxide and the leaf destruction on individual leaves varied from zero to as much as 100 per cent. in some cases. Also the clipping treatment was applied uniformly to all of the plants, whereas with the sulphur dioxide treatments the individual plants of the plot showed widely varying percentages of leaf destruction, as indicated by table II. It is interesting to note, therefore, the close agreement in the yields between corresponding percentages destroyed by fumigation and by clipping.

The clipping treatment was applied to the second crop only. The other crops throughout the season were harvested as checks. Nine treatments

were carried out as follows: single, double, and triple clippings, in each of which about 20, 50, and 90 per cent. of the leaves were removed from each stem. In the case of double and triple clippings, the 20, 50, and 90 per cent. respectively of the leaves on each stem at the time of each clipping were removed. About forty-five plants from five different plots were subjected to each treatment. These treatments were carried out in the following order:

1. First of the triple clippings
2. First of the double clippings
3. Single clippings
4. Second of the triple clippings
5. Second of the double clippings
6. Third of the triple clippings

The triple clipping was begun about ten days after the harvest of the first crop, and was finished about seven days before the harvest of the second crop. The leaves from each plant were dried and weighed separately. The results are shown in table V, which gives the yield of the second crop with and without the leaves that were removed, and also the yield of the other three crops.

TABLE V

EFFECT ON YIELD OF IMMEDIATE AND OF TWO SUBSEQUENT CROPS OF ALFALFA OF CLIPPING OFF DIFFERENT PERCENTAGES OF LEAVES; CLIPPINGS MADE FROM SECOND CROP

No. of plants	Second crop treatment		Yield in percentage of check (first crop)				
				Second crop			
	No. of clippings	Percentage of leaves removed	First crop	Weight	Weight + weight of leaves removed	Third crop	Fourth crop
45	1	20	100	94.2 ± 2.26*	98.7 ± 2.40*	104.2 ± 3.17*	96.0 ± 2.80*
42	1	50	"	80.3 ± 2.11	89.8 ± 2.34	97.5 ± 2.22	94.0 ± 2.87
45	1	90	"	68.0 ± 1.74	79.0 ± 1.89	93.5 ± 2.76	96.0 ± 2.68
48	2	20	"	85.5 ± 2.38	94.8 ± 2.47	100.6 ± 3.04	96.9 ± 2.54
45	2	50	"	70.9 ± 1.68	88.2 ± 1.74	91.9 ± 1.94	99.1 ± 2.46
46	2	90	"	57.8 ± 1.70	80.3 ± 2.50	81.6 ± 2.15	93.7 ± 2.97
48	3	20	"	70.4 ± 1.85	81.0 ± 2.12	99.6 ± 3.15	100.3 ± 2.73
47	3	50	"	61.3 ± 1.60	78.3 ± 1.94	93.5 ± 1.62	97.0 ± 2.25
46	3	90	"	41.5 ± 1.24	62.0 ± 1.77	73.8 ± 2.38	93.5 ± 2.15

* Standard error of the mean.

In figure 5 the yield data for the clipping treatments have been plotted and the curves superimposed upon the fumigation yield curves from figure 2. The close agreement between these curves indicates that correspond-

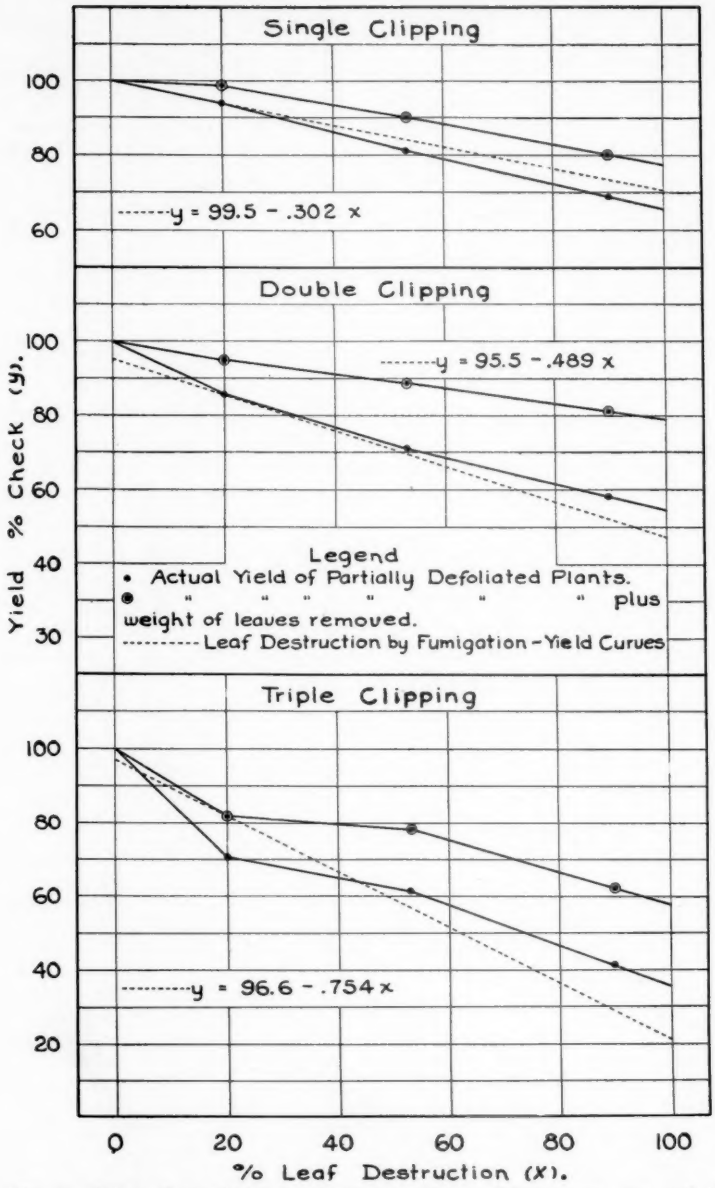

FIG. 5. Effect of clipping off various percentages of leaves one, two, and three times during the growth of a single crop on the yield of alfalfa. Treatments were applied on the second crop. The yield of each partially-defoliated crop is compared with the yield of the untreated crops from the same plots. Dotted lines are fumigation yield curves from fig. 2; 1931 data.

ing partial defoliations by clipping and by fumigation with SO_2 give approximately the same reduction of yield. It should be noted that in the case of light fumigations there is little tendency to shed the injured leaves, and consequently the abscission yield data plus the weight of the leaves removed should agree more closely with the fumigation yield data at small percentages of leaf destruction. On the other hand, the abscission yield data without the weight of the removed leaves should agree more closely with the fumigation data showing large percentages of leaf destruction, on account of the tendency to shed the badly marked leaves. This agreement is shown in figure 5. The most extreme treatments appear to reduce the yield somewhat more in the case of fumigation than in the case of corresponding defoliation by clipping. The difference, although small, is possibly due to an added percentage of green tissue which is lost to the plant when the badly burned leaves are shed. The remarkable concordance between the fumigation and abscission treatments indicates that sulphur dioxide affects the alfalfa plant essentially and proportionately by removing leaf tissue. If any other effect is produced, it is of a definitely minor character.

E. Effects of partial defoliation on subsequent crops.—Table V also indicates that the yield on the third and fourth crops, subsequent to clipping the leaves on the second crop, is nearly normal in the case of the less severe treatments. The yield of the third crop, however, following the triple 90 per cent. clipping on the second crop, is only about 74 per cent. of normal, and the yield of the fourth crop is only about 94 per cent. of normal. In the case of the double 90 per cent. clipping on the second crop, these values are 82 per cent. normal for the third crop and 94 per cent. normal for the fourth. This effect, which possibly is to be explained by root depletion during second-crop recovery, has also been similarly noted in the case of heavily fumigated treatments (table VI). The fumigation

TABLE VI

Influence of severity of sulphur dioxide fumigations upon relative yields of the subsequent crop of alfalfa

No. of plots	Average yield of fumigated crop		Average yield of subsequent untreated crop
	Range	Average	
	%	%	%
28	80–90	85.7	99.2 ± 3.7*
10	70–80	76.6	92.2 ± 6.7
8	60–70	65.8	81.3 ± 4.5
5	40–60	52.2	72.0 ± 7.5

* Standard error of the mean.

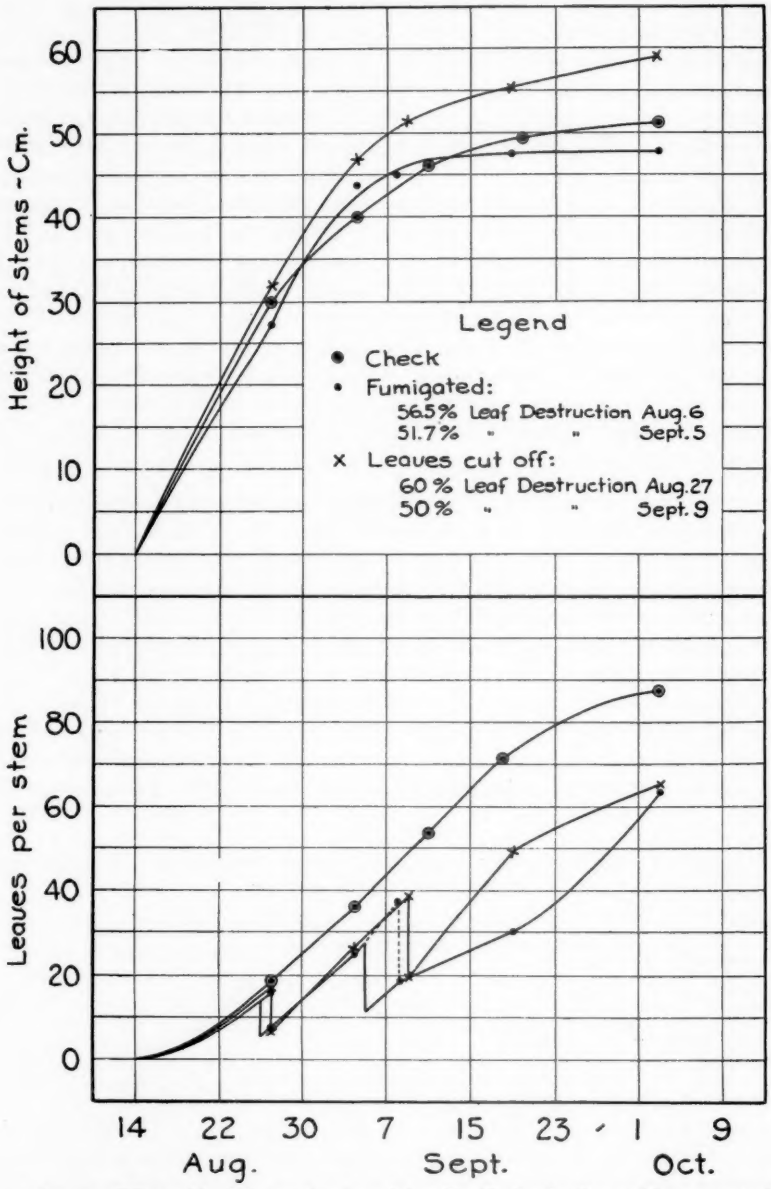

FIG. 6. Effect of leaf destruction by sulphur dioxide fumigation and by corresponding leaf clipping on the growth and formation of new leaves on individual stems of alfalfa.

treatments, however, were not especially planned to reveal this effect, as were the abscission treatments, and data are available on only one crop subsequent to the fumigation.

In table VI the 1930 yield data of the fumigated plots have been arranged in groups of 10 per cent. range, except for the fourth entry, in which it is 20 per cent. Tables V and VI indicate that the rates of recovery from abscission and fumigation treatments are nearly identical, and that the subsequent crop is practically normal if the treatment of the crop which precedes it does not reduce the yield more than about 15 per cent.

F. Growth and leaf production of individual alfalfa stems.—To determine the behavior of alfalfa plants following fumigation and clipping treatments, twenty comparable vigorous stems were twice subjected to each treatment and their subsequent growth was compared with that from forty corresponding untreated stems. In this experiment the numbers of leaves were counted and the heights of the stems were measured at intervals throughout the growth period. These data are presented in figure 6. At harvest time the average dry weight of the fumigated stems was 9.8 grams, of the clipped stems, 10.1 grams, and of the untreated stems, 16.2 grams. While these observations have not been sufficiently extensive in character to have quantitative significance, they indicate that recovery of the plants from fumigation treatments is similar to that from abscission treatments, and that in both cases the subsequent production of leaves and growth of stems is comparable with that of untreated stems.

Summary

1. Sulphur dioxide lesions on alfalfa are of two types, acute and chlorotic. The former shows characteristic bleached interveinal and marginal areas in otherwise normally appearing leaves; the latter exhibits a more or less yellowed and mottled appearance and is similar to chlorosis as produced by a number of other causes.

2. The reduction in yield of alfalfa subjected to a single sulphur dioxide fumigation of one crop is in direct proportion to the percentage of leaf area destroyed. The stage of growth at which the fumigation occurs does not appear to influence the result, at least within the range of 25–75 per cent. of the total growth period of the plant.

3. If a crop is fumigated more than once, assuming at least a week to elapse between fumigations each of which produces the same percentage of leaf destruction, the reduction of the yield is also in proportion to the number of fumigations.

4. The reduction in yield is not a linear function of the number of leaves marked, indicating that the uninjured portions of the leaves continue to function in spite of bleached areas.

5. The yield-leaf-destruction curves all closely approach 100 per cent. yield at 0-per cent. leaf destruction, indicating that the gas does not reduce the yield unless it produces visible effects.

6. The reductions of yield caused by sulphur dioxide fumigation can be closely duplicated by clipping off from normal plants an amount of leaf tissue equivalent in area to that destroyed by fumigation.

7. Like acute markings, chlorotic sulphur dioxide markings appear to lower the yield in direct proportion to the percentage of leaf tissue visibly affected.

8. A severe defoliation treatment, either by fumigation or by clipping, which lowers the yield of the treated crop extensively, also reduces the yield of the subsequent untreated crop appreciably, and may even be felt slightly in the second subsequent untreated crop.

9. The subsequent growth of new leaves, and the elongation of the stems following a partial defoliation, proceed at the same rate, regardless of whether the leaf destruction was accomplished by sulphur dioxide fumigation or by clipping.

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PHOTOSYNTHESIS IN VARIOUS PORTIONS OF THE SPECTRUM[1]

G. RICHARD BURNS

(WITH THREE FIGURES)

Introduction

The work on photosynthesis in various parts of the spectrum has been recently reviewed (**5, 1, 9**), and need not be repeated here except to call attention to the experimental methods and the results of the work of WARBURG and NEGELEIN (**6**), WURMSER (**7, 8**), and BRIGGS (**1**). Using a unicellular alga, *Chlorella vulgaris*, WARBURG and NEGELEIN found, under the conditions of their experiments, that for the reduction of one molecule of carbon dioxide, 4.4 quanta were necessary in the red, 4.3 in the green, and 5.1 in the blue. WURMSER found a higher value in the green, and concluded that photosynthesis does not follow the photochemical equivalence law. BRIGGS, working with land plants, agrees with WARBURG rather than with WURMSER. In the blue his results are similar to those of the writer in the blue-violet. WARBURG and WURMSER, using water suspensions of algae, were able to obtain a condition of complete absorption and very low reflection. BRIGGS, using cut leaves from land plants, had incomplete absorption. All corrected for respiration by adding the dark-rate respiration to their apparent rate of photosynthesis. This expedient is necessary if one wishes to determine the absolute quantum yields. In determining relative quantum yields, the writer's method makes it necessary to assume only that respiration is the same under two light intensities that are causing the same amount of photosynthesis.

The experiments reported in this paper were undertaken from a somewhat different point of view than the preceding. The chief object was to determine how efficiently certain trees could use various portions of the spectrum in photosynthesis. For this reason interest centered primarily on the incident radiation rather than on the absorbed radiation, and no attempts were made to measure absorption and reflection. To determine the photosynthetic efficiency on the basis of incident light, the plants could not be allowed to grow for a long time in colored light, as the effect of different wave lengths of light on the physical form of the plant entirely masks the photosynthetic effect (**2, 4**). Single leaves or groups of leaves could not be used, as the removal of any portion of a plant affects the rate of photosynthesis or of respiration. Narrow-leaved plants were used since

[1] Published with the consent of the Director of the Vermont Agricultural Experiment Station.

with broad-leaved plants a slight movement of the leaves may result in a considerable change in their effective area and reflection. The narrow-leaved plants used had the additional advantage that they were dark in color and thus absorbed a large portion of the incident radiation. The main features of the experimental method were: careful preliminary adjustment of the plant to the light intensity to be employed; use of light of constant and known intensity so adjusted that the amount of photosynthesis in each pair of determinations was the same; and, finally, measurement of the amount of photosynthesis from the decrease in carbon-dioxide concentration in the air surrounding the plant.

Apparatus

PLANTS USED.—White pine (*Pinus strobus*), Norway spruce (*Picea excelsa*), and Engelmann spruce (*Picea engelmanni*) were used, four-year-old nursery stock of regular shape and similar size being selected. They were potted and placed in a greenhouse three months before the work was begun.

The experiments were carried out in a constant-temperature room cooled by cold air and kept at the necessary temperature by a thermostatically controlled, electrical "space heater." The temperature at the plant was $28° \pm 0.5°$ C. unless otherwise stated. The temperature coefficient at this temperature, with the light intensities used, is one.

The light sources were four 1000-watt, 115-volt, projection type Mazda lamps operated at 110 volts. This type had the advantage of giving more light and having smaller bulbs than the ordinary lamp. The rate of decay in visible radiation was about 1 per cent. per hour at 110 volts and 2 per cent. at 115 volts. Line voltage fluctuations were virtually eliminated by the use of a General Electric miniature automatic induction voltage regulator. If the voltage fell to 109.5 or rose to 110.5, this instrument returned it to 110.0. This made the maximum deviation in light intensity about 1 per cent. and the average variation about 0.5 per cent. It was essential that some means of regulating the voltage be used, inasmuch as a 5.5 per cent. change in voltage resulted in a 16 per cent. change in the intensity of the radiation between 1150 mμ and 400 mμ, and a 28 per cent. change between 550 mμ and 400 mμ. To avoid reflected radiation, the bulb was surrounded with a blackened shield having a 10-cm. aperture toward the plant. A water filter was placed directly outside this aperture at a distance of 6 cm. from the filament. The water filter consisted of a 6-inch brass ring, 1 inch wide, with 3-mm. plate glass windows ground on and held in place by clamps. Tap water was circulated through these at 2.5 ft.^{3} per hour. It removed infra-red radiation to 1150 mμ. Because of the large area to be illuminated and the high intensity required, it was not pos-

sible to use anything approaching monochromatic light. Glass color filters were used which isolated the red, red-yellow, and blue-violet portions of the visible spectrum (fig. 1). These were fastened to the water cells. The blue-violet filters sometimes broke in spite of protection afforded by the water cell.

The radiation intensity was determined by a linear thermopile fitted with a 10-cm. water cell with quartz windows. A balanced-current method

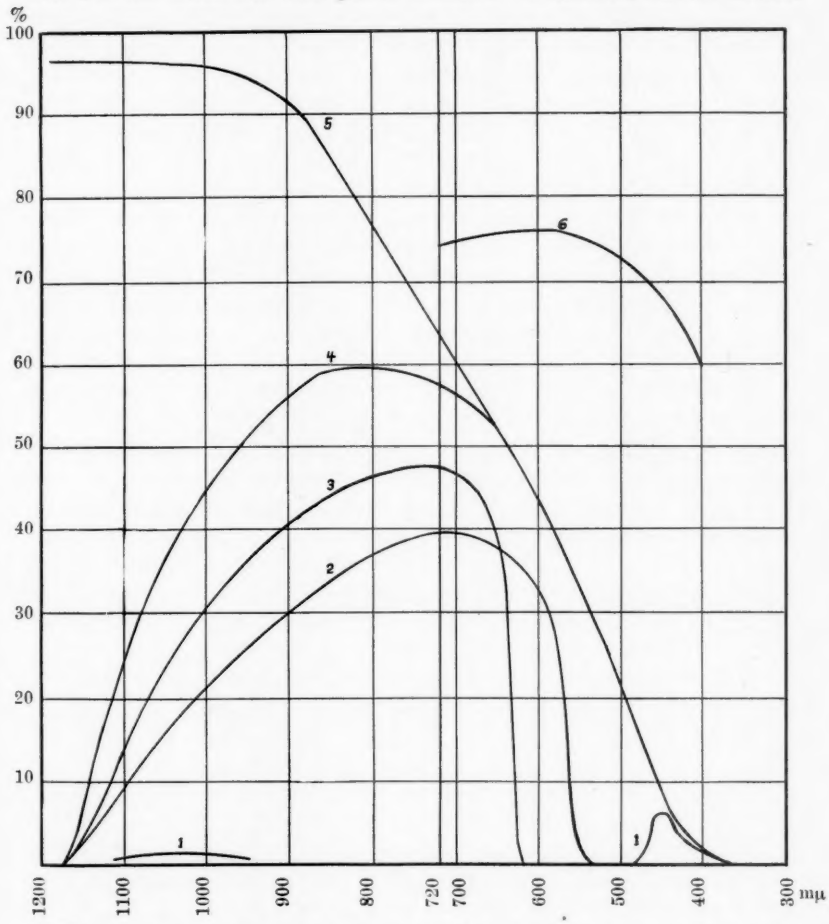

FIG. 1. Transmission spectra of filters with lamp at 110 volts. 1. Blue-violet + water filter. 2. Red-yellow + water filter. 3. Red + water filter. 4. Water filter. 5. No filter. 6. Sunlight (visible only, greatly reduced).

was employed, using a galvanometer as a zero instrument (3). The 10-cm. water cell insured more accurate readings by absorbing re-radiation from the hot glass filters; and, by absorbing an additional amount of the infra-red, made possible a more accurate calculation of the amount of visible radiation. The conversion factors necessary to determine the total radiation and the light intensity were found both by direct and by spectrometric

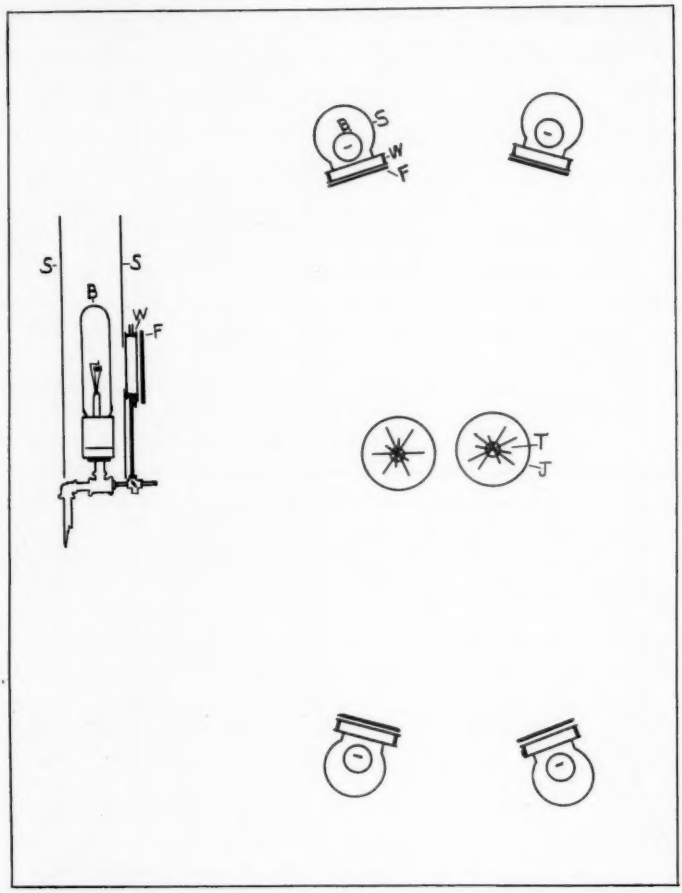

FIG. 2. Arrangement of lights, top and side view. B, bulb; S, shield; W, water filter; F, color filter; T, tree; J, bell-jar.

measurements. The factors by the two methods differed by not more than 0.4 per cent. The transmission spectra of the water cells and the color

filters were measured by means of a Hilger spectrometer (D 77) and thermopile.

The four lights were placed at the four corners of a rectangle, with one plant near the center and the second plant as close to it as possible (fig. 2). The plants were always set in exactly the same position, and when the lights were moved they were moved directly towards or away from the plant near the center. The radiation intensity was measured at the point that had been occupied by the center of the crown of the plant. This gave a value that is about 1 per cent. low, owing to the fact that some of the leaves are in front of this point and some are behind it. The lights and the trees were on the same level.

Since the relative amount of photosynthesis was determined by carbon-dioxide assimilation, generally considered the most accurate criterion, it was necessary to have the crown of the tree in a gas-tight container. This was done by fitting a ground-glass plate around the lower part of the stem and sealing a bell-jar on the plate with stop-cock grease. The bell-jar was equipped with a fan, so arranged that it would not shade the tree yet would provide adequate mixing of the gas before analysis. The smallest possible bell-jar was used, as this made the percentage decrease in carbon dioxide greater and thus increased the accuracy of the results. The bell-jars were used with the inside surface wet with a dilute soap solution. This was the only method found which would prevent the formation of a fog by the transpired moisture from the plant. The soap film was found to have no measurable effect on the carbon-dioxide concentration. The light transmission of the wet bell-jars was measured with the jar whirling rapidly with an excentric motion. In table I are presented the volumes and transmission data for the bell-jars used.

The carbon-dioxide determinations were made with a modified Haldane

TABLE I

Bell-jar	Runs used	Volume	Transmission
1	NS 1 1–68	8,850	89
1	P 1 All	.....	.....
2	NS 2 69–73	10,350	84
3	NS 2 1–68	10,750	88
4	ES 4 All	2,700	89.5
5	ES 3 All	5,150	90
5	P 4 All	.....	.....
5	P 3 41–43	.....	.....
6	P 3 1–41, 44–70	4,600	89
7	NS 1 69–73	5,260	88

gas analysis apparatus with which it was possible to determine the percentage of carbon dioxide to 0.005.

Experimental procedure

The crown of the tree was carefully inserted through a hole in a ground-glass plate and the opening around the stem closed with a rubber stopper and grafting wax. The whole was then set on a base which supported the pot and the plate so that they could not move. The tree was then placed outdoors in light of approximately the same intensity as was to be used in the experiment and left for one week. This was a most important precaution, as in the case of the spruces a decrease as great as 15 per cent. resulted in the amount of carbon dioxide used when the tree was exposed to bright sunlight the day before the determination. In the case of white pine, which is more sensitive to such changes, the minimum light requirement, that is, the light intensity at which photosynthesis and respiration balance, was found to have decreased 50 per cent. after the tree had been in dull light for six days.

For a determination, the plant was brought into the constant temperature room, the bell-jar sealed on and tested for leaks, carbon dioxide introduced until its concentration was about 1 per cent., and the plant placed in a light intensity near its minimum light requirement. After half an hour the gas was well mixed, an accurate analysis made, and the plant placed in the desired light intensity for exactly two hours. At the expiration of this time it was withdrawn, the radiation intensity measured, and the final gas analysis made. As a rule two plants were used at the same time and two sets of determinations were made each day. After the last analysis of the day the plants were again placed outdoors in the dull light.

As would be expected, it was found to be important that the initial amount of carbon dioxide be kept uniform. Initial concentrations as high as 2 per cent. and as low as 0.1 per cent. were found to make detectable differences in the minimum light requirement. The decrease in concentration of carbon dioxide during a determination is the amount that has been used by photosynthesis minus the amount given off by respiration. Since it was not possible to determine the amount of respiration, the light intensities were so adjusted that the total change in carbon dioxide was about the same in each pair of determinations, in order that the efficiency of the two kinds of light could be judged from their relative intensities. Under these conditions it was safer to assume that respiration, and therefore photosynthesis, was the same. The method had the additional advantage that the ranges of available carbon-dioxide concentrations were the same. The small corrections necessary to find the light intensities that would give exactly equal decreases in carbon-dioxide concentration were determined by

making a graph of the light intensities against the decrease in carbon dioxide (fig. 3). To eliminate errors due to the plants being imperfectly adjusted to the low light intensity, the determinations with colored light were run first one day and second the next. If the photosynthetic efficiency were still changing, the two sets of determinations would not agree but their average would be close to the true value.

Results

In the following tables the run number is the serial number of the day on which the determination was made, and the number of primes indicates whether it was the first, second, or third determination on the tree that day. The second column gives the number of the tree used: *P* for white pine, *NS* for Norway spruce, and *ES* for Engelmann spruce. The next column gives the filters used; the next, the decrease in carbon-dioxide concentration expressed as percentage of total gas; then the total radiation in calories per cm.^{2} per run $\times$ 10. The column headed "light" is the amount of radiation shorter than 720 mμ in calories per cm.^{2} per run $\times$ 10 (70 in these readings is about equal to 1 per cent. full sunlight). The values are not corrected for transmission of the bell-jars. The column "efficiency based on radiation" gives the number of parts of total radiation (see fig. 1) through the 1-inch water filter necessary to produce the same decrease of carbon dioxide as 100 parts of the radiation being studied. The next column is the same but based on the light intensity and not the total radiation. For example, in table II, white pine 3 on an afternoon run in the 43rd experiment with the radiation from the bulbs filtered through 1 inch of water and the blue-violet filter reduced the percentage of carbon dioxide in the bell-jar by 0.065 and 0.060, as shown by duplicate readings. During this time the total radiation received by the plant was 58.8 cal. per cm.^{2} $\times$ 10, and the light, that is, the amount of radiation shorter than 720 mμ, was 40.0 cal. per cm.^{2} $\times$ 10. When the blue-violet filter was removed and the lights moved farther away, however, the same tree in the noon run (43″) reduced the percentage of carbon dioxide in the bell-jar by 0.085, 0.080. The total radiation in this determination was 57.2 and the light 19.2. The amounts of radiation and light necessary to reduce the percentage of carbon dioxide by 0.0625, determined by means of a graph (fig. 3), were 48.0 and 16.2 respectively. The calculated value for the radiation through the 1-inch water filter multiplied by 100, divided by the amount of the blue-violet radiation, gives 81.7, the efficiency of the blue-violet radiation. That is, 81.7 parts of radiation through the 1-inch water filter were equal to 100 parts of radiation through the 1-inch water filter plus the blue-violet filter. Taking as values the light intensities rather than the total radiation intensities, the efficiency of the blue-violet is 40.4. These figures are based

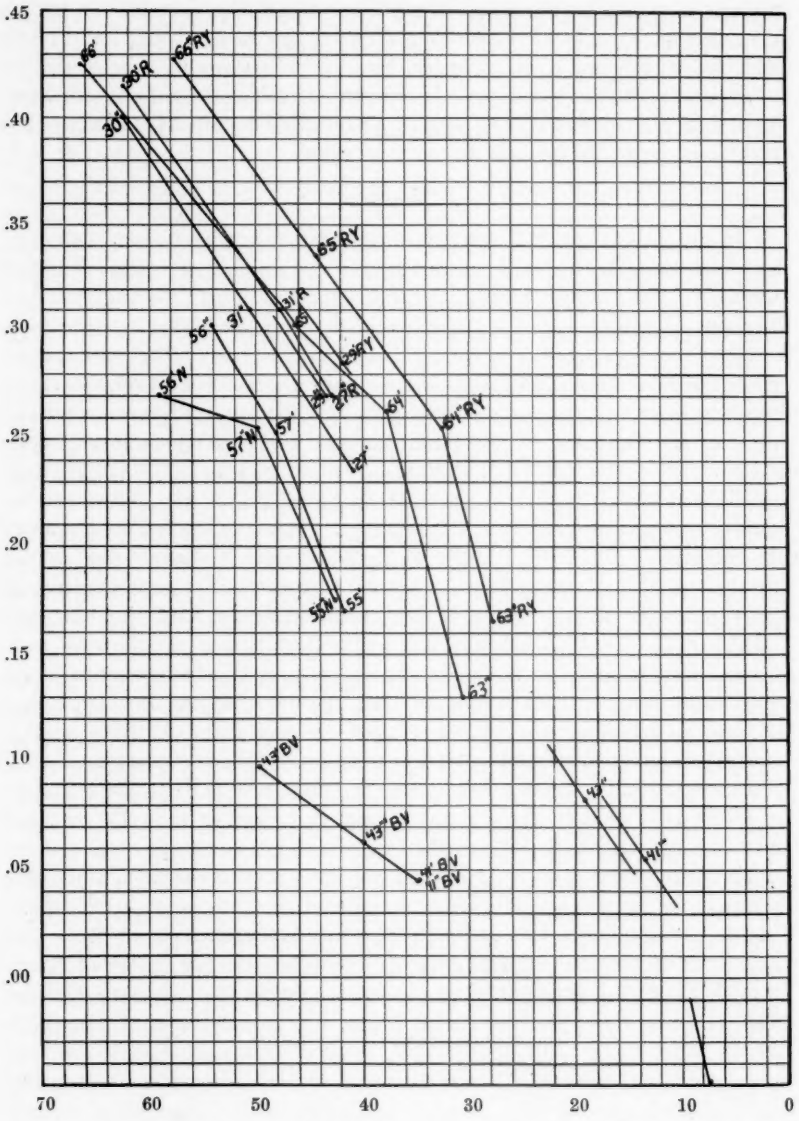

FIG. 3. Curves of decreased percentage of carbon dioxide plotted against light intensity. White pine 3. N = no filters. Run numbers alone indicate 1-in. water filters. R = 1-in. water filter + red filter. RY = 1-in. water filter + red-yellow filter. BV = 1-in. water filter + blue-violet filter.

on the assumption that radiation of wave length longer than 720 mμ is useless in photosynthesis. This is a major assumption, of course, and practically without direct experimental proof. There are, however, many things indicating that this is true. An investigation of the exact long wave-length limit of photosynthesis will be started in the near future. The final column gives the sum of the errors that would result from an error of 0.01 per cent. (twice the actual error) in the gas analysis, and the probable error in correcting the light intensity from the graph. In addition to this there are errors of 1 per cent. in measuring the radiation intensity, 0.5 per cent. from variation of the radiation intensity, and a constant error in calculating the radiation and light intensity.

In the runs in which the blue-violet filter was used, the voltage was raised to 115 in order to increase the amount of energy in this portion of the spectrum. The runs with the water filter alone were at 110 volts. Owing to the low intensity of the blue-violet, the lights were much closer to the plant, and this slightly increased the error in measuring the light intensity. To reduce the white light to a comparable intensity, it was necessary to use smoked screening in front of the water cells in addition to moving the lights farther away. It should be noticed that the change in efficiency in white pine 4 is due to a loss of efficiency in white light, rather than to any change in the blue-violet. For this reason white pine 3 was given a 7-hour exposure to the blue light in run no. 42. In this case no such marked change was observed.

The efficiency of the red-yellow would be the same regardless of the long wave-length limit of photosynthesis, since these filters transmit the same proportions of infra-red as do the 1-inch water cells. White pine 4 gives the same results here that it did with the blue-violet. With white pine 3 a slight fog was formed as noted. This throws the values off by as much as 10 per cent. Determinations 65 and 66 are probably the most accurate, as the 64′ falls out of place on the graph.

The determinations 58 to 60 inclusive, on white pine and Engelmann spruce, were made after the trees had been placed in a small cubical greenhouse glazed with 80 panes of the red filter glass and left for three weeks in full sunlight. The last ten days of this time were almost cloudless. The customary acclimation period was omitted. It will be noticed that this treatment resulted in a considerable change in the relative efficiencies.

These determinations show that infra-red radiation longer than 1100 mμ is slightly detrimental to photosynthesis when the plant is at a sufficiently high temperature. It should be noted that the temperature coefficient of photosynthesis at 28° C. is so near 1 that no effect of temperature can be detected in these measurements. It is also interesting to note

TABLE II

BLUE-VIOLET

Run no.	Tree no.	Filters	Decrease in CO_2		Total radiation	Light	Efficiency of blue-violet based on		Experimental error
			I	II	Cal. per cm.^{2} × 10		Radiation	Light	
			%	%	*cal.*	*cal.*	%	%	%
33′	ES 4	1″	0.110	0.110	66.6	22.4			3.0
36′	ES 4	1″–BV	0.050	0.050	69.2	47.1	71.0	35.2	3.0
36″	ES 4	1″	0.060	0.060	57.4	19.3*			
37′	ES 4	1″–BV	0.070	0.065	80.0	54.4	67.9	33.6	3.0
37″	ES 4	1″	0.055	0.060	52.3	17.6			
37‴	ES 4	1″–BV	0.045	0.045	66.2	45.0	71.0	35.2	3.0
41′	P 3	1″–BV	0.045	0.045	51.2	34.8	70.5	34.8	4.5
41″	P 3	1″–BV	0.050	0.040	51.3	34.9			
41‴	P 3	1″	0.055	0.055	40.3	13.6	73.0	36.2	6.0
42	P 3	1″–BV	.....	.....	44.1	30.0	.....	.....	.....
43′	P 3	1″–BV	0.095	0.100	73.0	49.7	86.7	42.7	3.0
43″	P 3	1″	0.085	0.080	57.2	19.2			
43‴	P 3	1″–BV	0.065	0.060	58.8	40.0	81.7	40.4	3.0
38′	P 4	1″–BV	0.100	0.100	64.0	43.5	84.0	41.5	5.0
38″	P 4	1″	0.065	0.065	42.7	14.4			
38‴	P 4	1″–BV	0.115	0.115	64.8	44.1	89.0	44.0	5.0
38⁗	P 4	1″	0.080	0.075	55.9	18.8	100.0	49.5	4.0
39″	P 4	1″	0.100	0.095	68.3	22.9	112.0	55.5	4.0

* Fog 37″ value used.

TABLE III

RED-YELLOW

TABLE III

RED-YELLOW

Run no.	Tree no.	Filters	Decrease in CO_2		Total radiation	Light	Efficiency of red-yellow based on		Experimental error
			I	II	Cal. per cm.^{2} × 10		Radiation	Light	
			%	%	*cal.*	*cal.*			%
29′	P 4	1″–RY	0.355	0.340	134.0	45.4	99.3	98.7	2.0
29″	P 4	1″	0.335	0.335	128.0	43.4			
63′	P 4	1″–RY	0.135	0.130	78.7	26.7	92.2	91.6	3.0
63″	P 4	1″	0.140	0.140	75.5	25.4			
64′	P 4	1″	0.240	0.240	111.0	37.4	112.3	111.7	2.0
64″	P 4	1″–RY	0.235	0.235	96.3	32.7			
65′	P 4	1″–RY	0.300	0.305	123.0	41.9	123.6	122.9	2.0
65″	P 4	1″	0.265	0.270	135.0	45.6			
66′	P 4	1″	0.305	0.305	187.0	62.9	151.3	150.5	3.0
66″	P 4	1″–RY	0.400	0.395	164.0	55.6			
29′	P 3	1″–RY	0.290	0.280	123.0	41.9	110.1	109.5	2.0
29″	P 3	1″	0.270	0.270	129.0	43.4*			
63′	P 3	1″–RY	0.165	0.165	82.2	27.8*	111.2	110.2	5.0
63″	P 3	1″	0.130	0.130	91.3	30.7*			
64′	P 3	1″	0.265	0.260	111.0	37.3	112.0	111.0	2.0
64″	P 3	1″–RY	0.255	0.255	96.2	32.6			
65′	P 3	1″–RY	0.335	0.335	131.0	44.4	117.5	116.8	1.5
65″	P 3	1″	0.305	0.300	138.0	46.4			
66′	P 3	1″	0.430	0.420	197.0	66.5	117.0	116.3	1.0
66″	P 3	1″–RY	0.425	0.430	170.0	57.7			
69′	NS 1	1″–RY	0.400	0.400	193.0	65.4	106.0	105.3	3.0
69″	NS 1	1″	0.440	0.440	232.0	78.2			
72′	NS 1	1″	.....	.....	.....	.....	.....	.....	.....
72″	NS 1	1″–RY	0.375	0.370	159.0	53.7			
73′	NS 1	1″–RY	0.435	0.435	202.0	68.6	104.7	104.0	3.0
73″	NS 1	1″	0.420	0.420	201.0	67.8			
69′	NS 2	1″–RY	0.210	0.205	191.0	64.7	102.6	102.0	4.0
69″	NS 2	1″	0.245	0.245	226.0	76.2			
72′	NS 2	1″	0.225	0.225	180.0	60.6	106.3	106.0	4.0
72″	NS 2	1″–RY	0.200	0.200	155.0	52.4			
73′	NS 2	1″–RY	0.220	0.215	196.0	66.5	102.5	102.0	4.0
73″	NS 2	1″	0.220	0.215	201.0	67.7			

* Slight fog.

TABLE IV

RED

Run no.	Tree no.	Filters	Decrease in CO_2		Total radiation	Light	Efficiency of red-yellow based on		Experimental error
			I	II	Cal. per cm.^{2} × 10		Radiation	Light	
			%	%	*cal.*	*cal.*			%
27′	P 3	1″	0.235	0.235	122.0	40.9	67.5	107.0	2.0
27″	P 3	1″–Red	0.270	0.270	204.0	42.9			
30′	P 3	1″–Red	0.415	0.415	298.0	62.5	65.0	104.5	6.0
30″	P 3	1″	0.800	0.795	373.0	125.2			
31′	P 3	1″–Red	0.310	0.310	227.0	47.8	65.9	106.0	2.0
31″	P 3	1″	0.310	0.310	150.0	50.6			
27′	P 4	1″–Red	0.300	0.300	132.0	44.2	65.5	105.4	2.5
27″	P 4	1″	0.295	0.295	197.0	41.4			
30′	P 4	1″–Red	0.450	0.445	285.0	59.8	65.5	105.4	6.0
30″	P 4	1″	0.765	0.750	346.0	116.0			
31′	P 4	1″–Red	0.380	0.390	226.0	47.6	67.0	107.0	2.0
31″	P 4	1″	0.385	0.380	150.0	50.6			
58′	P 1	1″–Red	0.400	0.400	242.0	50.7	.....	.....	.....
58″	P 1	1″	.....	.....	.....	.....	.....	.....	.....
59′	P 1	1″–Red	0.300	0.305	180.0	37.9	77.0	123.5	1.5
59″	P 1	1″	0.290	0.280	132.0	44.4			
60′	P 1	1″	0.350	0.350	160.0	53.8	71.5*	114.8*	.....
60″	P 1	1″–Red	0.305	0.305	196.0	41.2*			
23″	ES 4	1″	−0.005	−0.005	42.2	14.2	65.7	106.0	5.0
23‴	ES 4	1″–Red	0.000	0.000	66.3	13.9			
24′	ES 4	1″	0.150	0.155	95.5	32.1	64.0	102.0	1.5
24″	ES 4	1″–Red	0.145	0.145	146.0	30.7			
23″	ES 3	1″	0.005	0.000	42.8	14.4	64.2	103.0	7.0
23‴	ES 3	1″–Red	0.000	0.000	64.8	13.6			
24′	ES 3	1″	0.155	0.150	100.0	33.7	65.9	106.0	2.0
24″	ES 3	1″–Red	0.155	0.160	155.0	32.2			
58′	ES 3	1″–Red	0.390	0.390	247.0	52.4	.....	.....	.....
58″	ES 3	1″	.....	.....	.....	.....	.....	.....	.....
59′	ES 3	1″–Red	0.305	0.305	191.0	40.3	75.4	120.0	1.0
59″	ES 3	1″	0.290	0.290	138.0	46.4			
60′	ES 3	1″	0.395	0.395	172.0	57.7	76.5	123.0	1.5
60″	ES 3	1″–Red	0.365	0.365	211.0	44.7			

* Fog value low.

TABLE V

* Fog value low.

TABLE V

INFRA-RED

Run no.	Tree no.	Filters	Decrease in CO_2 I	Decrease in CO_2 II	Temperature	Total radiation	Light	Efficiency of infra-red + light based on light		Experimental error
						Cal. per cm.^{2} × 10				
			%	%		*cal.*	*cal.*			%
55′	P 3	1″	0.175	0.165	29.5	124.0	41.8	99.0	92.3	1.5
55″	P 3	None	0.170	0.180	29.5	372.0	42.6			
56′	P 3	None	0.270	0.270	30.5	514.0	59.1			
56″	P 3	1″	0.305	0.300	30.0	161.0	54.1	85.5	91.7	1.0
57′	P 3	1″	0.250	0.255	29.5	143.0	48.1			
57″	P 3	None	0.255	0.255	29.5	434.0	49.9	97.6		1.0
55′	P 4	1″	0.205	0.205	29.5	127.0	42.6	96.0	91.7	1.5
55″	P 4	None	0.220	0.215	29.5	397.0	45.7			
56′	P 4	None	0.315	0.315	30.5	531.0	61.0			
56″	P 4	1″	0.345	0.345	30.0	169.0	57.0	87.3	90.1	1.0
57′	P 4	1″	0.275	0.270	29.5	144.0	48.7			
57″	P 4	None	0.270	0.260	29.5	450.0	51.7	92.8		1.0
13‴	NS 1	1″	0.000	0.000	27.0	29.4	9.9			10.0
44′	NS 1	1″	0.150	0.160	28.0	158.0	53.3	92.5		2.5
44″	NS 1	None	0.125	0.135	31.0	453.0	52.1			
45′	NS 1	None	0.340	0.340	33.5	974.0	112.0	87.5		1.0
45″	NS 1	1″	0.395	0.395	33.5	332.0	112.0			
53′	NS 1	None	0.210	0.210	29.5	561.0	64.5	92.2		2.0
53″	NS 1	1″	0.235	0.225	28.5	189.0	63.5			
54′	NS 1	1″	0.220	0.225	29.5	178.0	60.0	95.4		2.0
54″	NS 1	None	0.220	0.230	29.5	551.0	63.3			
14′	NS 2	1″	0.000	0.000	27.0	29.8	10.0	.....		10.0
44′	NS 2	1″	0.125	0.120	28.0	155.0	52.1	93.0		3.0
44″	NS 2	None	0.110	0.110	31.0	448.0	51.5			
45′	NS 2	None	0.285	0.300	33.5	914.0	105.0	96.6		1.5
45″	NS 2	1″	0.320	0.315	33.5	324.0	109.0			
53′	NS 2	None	0.165	0.170	29.5	503.0	58.0	.....		.....
53″	NS 2	1″	.....	.....	.....	.....	.....	.....		.....
54′	NS 2	1″	0.210	0.210	29.5	171.0	57.7	86.8		2.5
54″	NS 2	None	0.190	0.195	29.5	537.0	61.7			

that with pine the adverse effect of the infra-red is more noticeable in the early morning run than in the later one.

The following summary gives the amount, in calories, of white light (720–390 mμ) necessary to give the same percentage decrease in carbon-dioxide concentration as 100 calories of the light in question:

	NS 1	NS 2	ES 3	ES 4	P 3	P 4	ES 3 | P 1 ACCLIMATED	
Blue-violet	.....	.....	.....	34.4	38.8	39.5	.....	.....
Red-yellow	106	103	.....	.....	116.0	.....	.....	.....
Red	.....	.....	106	102.0	106.0	.....	121	123
Infra-red and white	91	92	.....	.....	92.0	91.0	.....	.....

Relative quantum yields

The effective area of white pine 3 was calculated by photographing the tree from the positions of the four lights, enlarging these, and cutting out the unexposed portions. The weight of these was then compared with the weight of a known area photographed and enlarged in the same manner. Suitable precautions were taken to avoid the creeping of the background over the image. The average of the four areas was 105 ± 10. cm.^{2}

The respiration of this plant was taken as 0.17 per cent. That is the amount that the plant respires in the two hours immediately following a run, and was close to the exterpolated value from the graph. After some hours in darkness it dropped to 0.04 per cent. Assuming complete absorption, no reflection, and the limit of photosynthesis at 720 mμ, the value varies from 8 quanta per molecule of carbon dioxide in the low light intensities to 12 in the high. Higher quantum yields could undoubtedly be obtained by longer acclimation of the plant to low light intensities. The loss by reflection and transmission was about 40 per cent. This would lower these values to 5 and 7.

The relative quantum yields are of more significance and are given in the following summary:

	NS 1	NS 2	ES 3	ES 4	P 3	P 4
Blue-violet	.....	.....	.....	47.5	53.5	54.3
Red-yellow	101	98	.....	.....	111.0	.....
Red	.....	.....	96.5	93.0	96.5	.....

The low values in the red may be due to the fact that photosynthesis stops before 720 mμ or to low absorption. The differences between the pine and the spruce seem larger than that which could be attributed to their difference in color. The very low values for the blue-violet must be due to an actual falling off of photosynthesis. The results with red-yellow also indicate this. Since white light was about 70 per cent. red-yellow, the efficiency of the remaining 30 per cent. must be low when 117 or 105 parts of white are necessary to equal 100 parts of red-yellow.

Summary

1. A method for determining the relative quantum yields in photosynthesis in different portions of the spectrum has been described and the experimental errors determined.

2. Infra-red radiation of wave length longer than 1100 mμ is detrimental to photosynthesis.

3. With spruce the relative quantum yields in the wave lengths 720–630, 720–560, and 470–390 mμ are 9.5, 10, and 5; and with pine, 9.5, 11, and 5.

4. The actual quantum yield with pine is between 5 and 12 quanta per mole of carbon dioxide.

5. A survey of these results from a botanical aspect will be published from the Vermont Agricultural Experiment Station, where this work was carried on. These experiments are being continued.

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EFFECT OF LIGHT ON THE BIOELECTRIC POTENTIALS OF ISOLATED *ELODEA* LEAVES

H. BENTLEY GLASS

(WITH NINE FIGURES)

Introduction

The theory that the electric polarities of cells and tissues of living organisms are probably due to oxidation-reduction systems has been advanced by LUND and his associates (**2**). They have further suggested that such potentials constitute a mechanism of correlation between the cells or parts of the organism (**2**). The effect of light upon such systems seems therefore worthy of investigation. In 1892 OTTO HAAKE showed that green leaves increase their E.M.F.s in a positive direction when illuminated, and that other plant tissues, if chlorophyll-free, are not so affected (**1**). Since that time methods for the detection of electrical phenomena have been greatly improved, but the question of the effect of radiant energy upon bioelectric phenomena in plants has remained practically unexplored. A summary of the work done up to the present upon these problems is to be found in the paper by WALLER (**7**). In the present paper the results of a study of the responses made by single isolated leaves of *Elodea* are reported. This material was selected because of its simplicity of structure, each leaf having only two similar layers of large rectangular cells, and only one vein, the midrib; and because the absence of a cuticle facilitates the measurement of the potentials.

Method

The potentials between the tips and basal ends of detached leaves were measured with a Compton quadrant electrometer, the electrical circuit being identical with the one described by LUND (**3**). Isoelectric saturated zinc sulphate electrodes were used, and contact with the leaves was made through glass contact cups and arms, containing tap water (fig. 1). The leaves were placed in a moist chamber covered by a hood to exclude the light. They were supported on the contact arms in an upright position, so that the basal end was connected to the grounded quadrants, and the apex to the opposite pair (fig. 1). Since diffusion through the capillary arms of the contacts was very slow, and the water in them was changed every day, there was no danger of zinc sulphate solution reaching the leaf and injuring it. The contact cups passed through holes in the base of the moist chamber. These openings were sealed with flexible rubber joints, permitting movement of the contacts from outside the chamber while maintaining its hermetic sealing. When the walls of the moist chamber were covered with wet

filter paper and the chamber was saturated with moisture, a leaf could be kept alive and in a normal state in it for many hours. The source of illumination was an electric globe with a linear filament, the beam being focused, after passing through a water chamber, by a microscope objective and ocular, then passing through a window into the moist chamber, and finally through the aperture of an iris diaphragm so as to fall upon the leaf

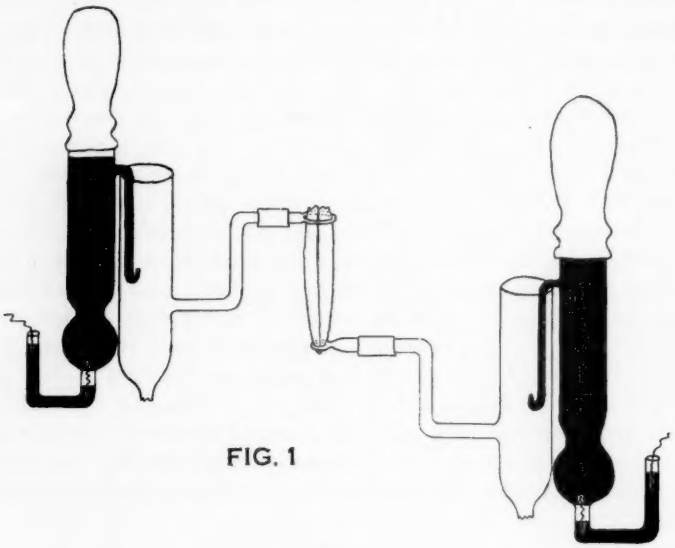

FIG. 1

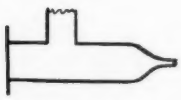

FIG. 1A

FIG. 1. Diagram of zinc sulphate electrodes, contact cups containing tap water, and contacts, with a leaf in position.

FIG. 1A. Special capillary contact for leading off at various points along surface of leaf.

in a small round spot 3 mm. in diameter, with an intensity of illumination of approximately 18,000 foot-candles. The light source was mounted upon a heavy base arranged to provide micro-manipulatory movement in three planes. All experiments were repeated at least three times, and more in the case of divergence. No two leaves react in precisely the same way, yet the general agreement is very good. Readings were plotted directly in graph form from the scale at 12-second intervals.

Absence of effect of light on electric polarity of onion root

Preliminary readings were made on onion roots, 4 to 6 cm. in length. These remained intact upon the bulb in a perfectly normal state, and continued growth following the experiments. The usual large inherent potentials were observed, which fluctuated when the roots were mechanically stimulated; but absolutely no effect followed illumination. This result confirms the observations of HAAKE (**1**) and WALLER (**7**), and apparently definitely confirms the relation of the phenomena to be described in *Elodea* to the presence of the chloroplasts.

Effect of light on electric polarity of *Elodea* leaf

The isolated leaves of *Elodea* were always dark-adapted, and allowed to remain in the moist chamber for a period varying from 40 minutes to 2 hours before illumination was begun. This was necessary in order that the effect of mechanical stimulation might subside, and that the potential might reach a stable level. This basal level of the electric polarity in a dark-adapted leaf was low, generally amounting to less than 10 millivolts between apex and base.

Experiments were conducted using both apical and basal leaves. By the former term is meant those leaves taken from a region within 2 inches of the growing tip, and of a light yellowish green color; by the latter term is meant one taken 10 or more inches from the tip, of a dark green color, and from a region where the whorls are spaced far apart.

Either apical or basal leaves, when placed in the moist chamber, showed that initially the apex was positive to the base. This E.M.F. was always found to be falling rapidly, as soon as observations could be made, and soon reached a level close to zero potential, either slightly above or below, depending upon the original condition of the leaf. If the hood was not

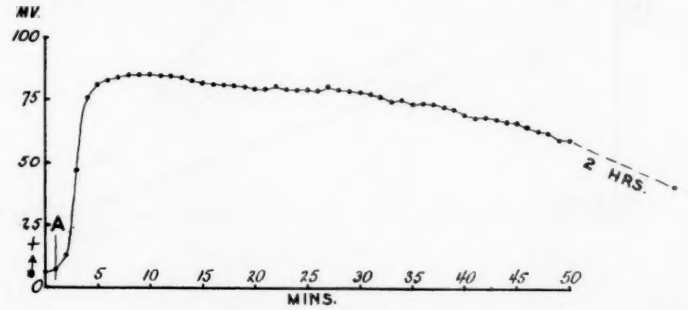

FIG. 2. Change of potential in an apical *Elodea* leaf under constant illumination of an apical spot; *A*, beginning of illumination.

placed over the leaf, it recovered to such a state that the apex again was positive to the base after a period of 1–2 hours, by an amount approximately equal to the originally observed potential. But when the leaf was placed in the dark, it found a new level of equilibrium, close to zero potential.

When a dark-adapted apical leaf of *Elodea* was illuminated by a round spot focused on the apex of the leaf, within a period of 5–10 minutes the potential between apex and base rose from nearly zero to a level between 75 and 100 millivolts (fig. 2). Under continuous illumination after the maximum response was attained, there was always found to be a slow

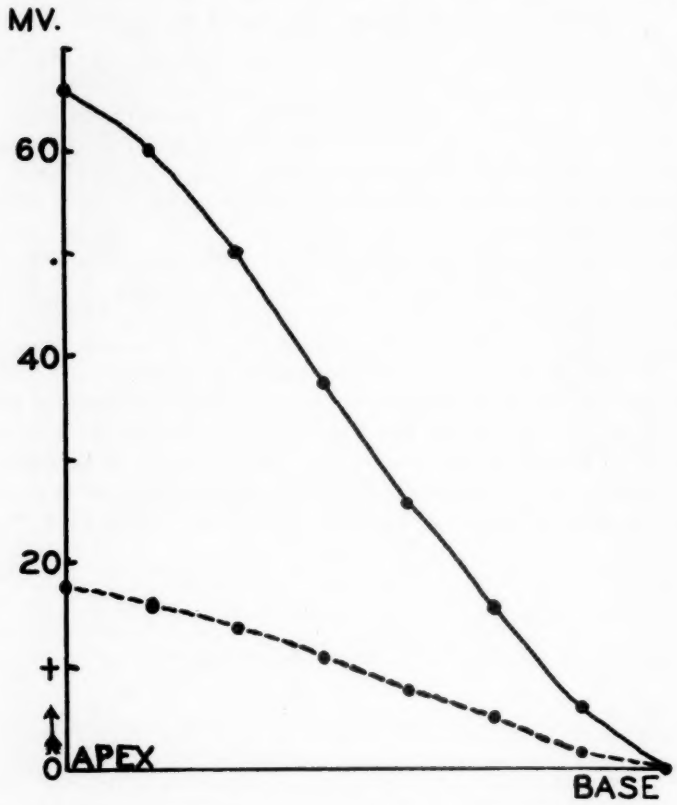

FIG. 3. Magnitude of E.M.F.s along an apical *Elodea* leaf, measured with respect to the base:

dotted line, when unillumined;
solid line, when illuminated at an apical spot.

decrease in the magnitude of the potential. After several hours, it was only about half as great as at first. When more than one reading was made upon the same leaf, the successive ones were always of decreasing magnitude. WALLER (**5, 6**) has reported some evidence of "fatigue" in his experiments upon the effects of light on the potentials of green leaves. This seems to be confirmatory, therefore, but should not be regarded as conclusive evidence, since the observations were only incidental to the main purpose in view, and the conditions were therefore not sufficiently controlled to that end.

It has been stated that the apex is positive to the base before illumination. But often both apex and base are positive to the middle portion of the leaf (fig. 4). Yet the decrease of E.M.F.s along the leaf may be uniform, and the base then the point of lowest potential (fig. 3). Illumination of the apex causes its potential to be greatly increased over that of the base; and an increase of potential also occurs along the entire leaf, diminishing with distance from the point of illumination. The decrease of E.M.F.s along the leaf is uniform, and appears as practically a straight line in the graph (fig. 3). This occurred in all cases, regardless of the original orientation of E.M.F.s along the leaf. When the leaf was illuminated at the basal end, the base became markedly positive to the apex, and again the transmission of the effect of non-illuminated parts of the leaf was observed.

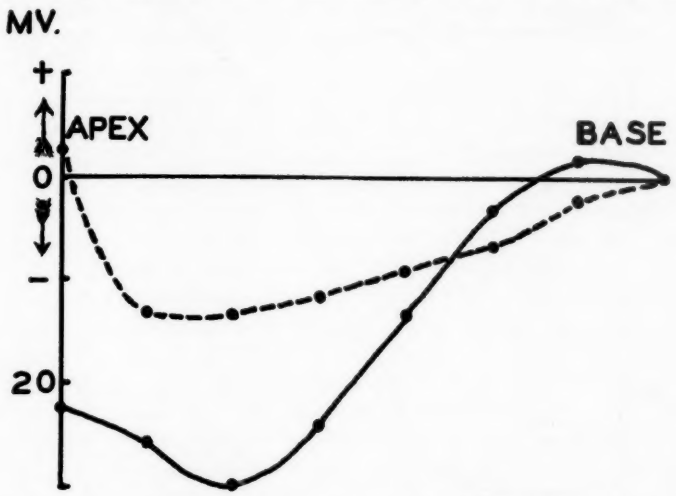

FIG. 4. Magnitude of E.M.F.s along an apical *Elodea* leaf, measured with respect to the base:

dotted line, when unillumined;
solid line, when illuminated at a basal spot.

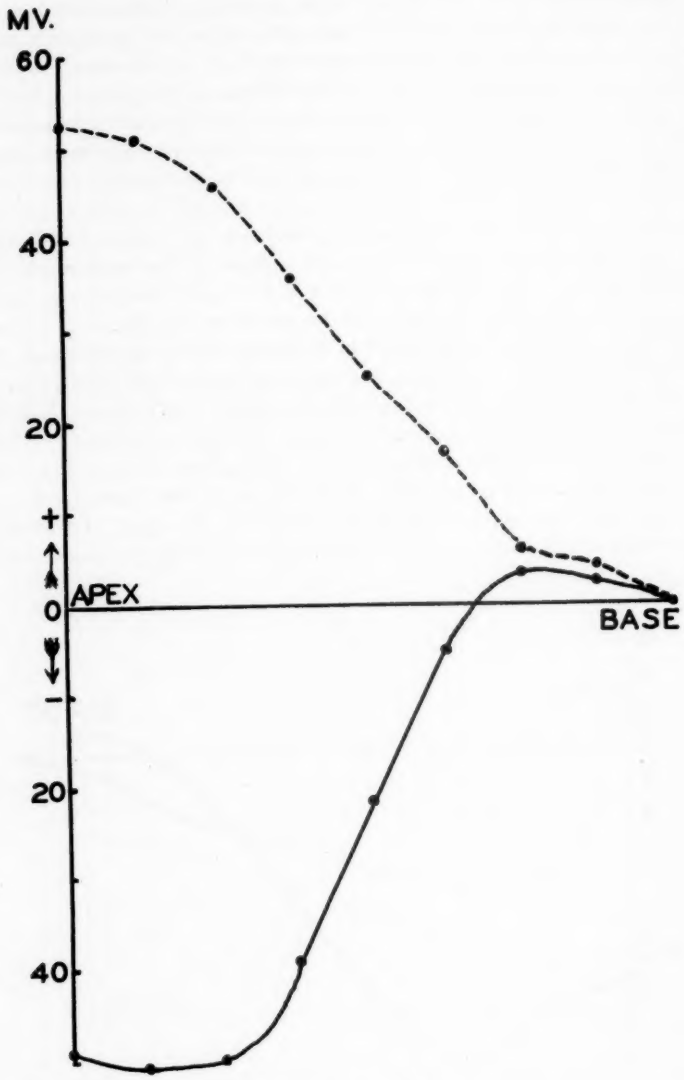

FIG. 5. Same as in figure 4.

This too occurred regardless of the original orientation of the E.M.F.s (figs. 4, 5). The solid lines on both these figures show, however, that there is a short region close to the tip of the leaf where the original orientation is preserved. It is further to be observed in these two figures that the E.M.F. is at a maximum at the point of application of the light, 2 to 4 mm. from the base. Again, when a point outside the positions of the contacts was illuminated, the region between the contacts being maintained in darkness, transmission of the effect of light to the region between the contacts was observed in the same manner. In plotting the magnitudes of the E.M.F.s along the leaf, the contact at the base was connected with the grounded quadrants, as before; while a new capillary tip contact was made which could be moved up or down at will, and also moved back from the leaf surface, thus avoiding stimulation of the leaf by motion of the contact along it; actual contact was made only through the drop of tap water at the tip (fig. 1A). Figures 4 and 5 show that although the base of the leaf was illuminated and the apex was maintained in darkness, yet apparently the potential at the apex has been greatly altered while that at the base has been maintained constant. It must be emphasized that this effect is only apparent, owing to the conditions of the experimental technique. It is due to the fact that, as in the experiment graphed in figure 3, the base of the leaf was connected with the grounded quadrants. What actually occurs is that, as all other evidence shows, the potential of the base is greatly increased when it is illuminated, while that of the apex, remaining in darkness, is relatively constant.

Apical leaves react to illumination in the typical manner shown in figure 6. Curve I represents the change of potential under constant illumination of a spot at the apex of the leaf; curve II represents the same change when a spot in the center of the leaf is illuminated; and curve III similarly for a spot at the base. The time at which the light was turned on is marked by the vertical line at *A*, and the time at which it was turned off by the arrow at *B* upon each curve. In each case the response is rapid, reaching the maximum in 7 to 10 minutes. As soon as the illumination ceases, the potentials promptly drop. The response when the apex is illuminated is always positive, *i.e.*, the potential of the apex as compared with that of the base is increased. It is large in magnitude. When the central portion is illuminated, the response is always positive but is much less in magnitude; when the base is stimulated the response is again large, but is in a negative direction, *i.e.*, the potential of the base with respect to that of the apex is increased. This response is sometimes even larger than the positive response when the apex is illuminated. It was also noted that in the second of the three cases the response varied considerably, being sometimes large and sometimes small. Twice positive responses were obtained, although of

slight magnitude, when the basal end was illuminated. These exceptions to the general case were probably due to poor material.

Basal leaves were also similarly illuminated (fig. 7). The magnitude of the responses, other conditions being the same, appear to be smaller than the responses in apical leaves.

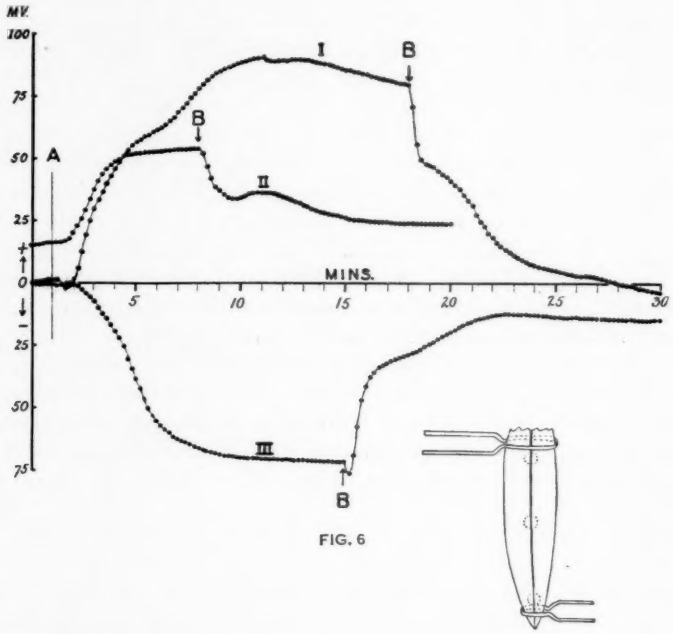

FIG. 6

FIG. 6A

FIG. 6. Change of potenial in an apical *Elodea* leaf under illumination:
I, at apical spot (see fig. 6A);
II, at spot in center of leaf;
III, at basal spot.
A marks the instant at which illumination began; *B* the one at which, in each case, it ceased.

FIG. 6A. Diagram of an *Elodea* leaf in position on contacts. The circles mark various positions illuminated.

Examination of the individual curves (figs. 6, 7) shows certain constant features. There is a slight lag of about a minute after the illumination begins before any response can be observed. The initial response is sometimes a change in the opposite direction to that in which the succeeding main response occurs. The steady decrease after maximum response is attained has already been mentioned. When illumination ceases, there is a

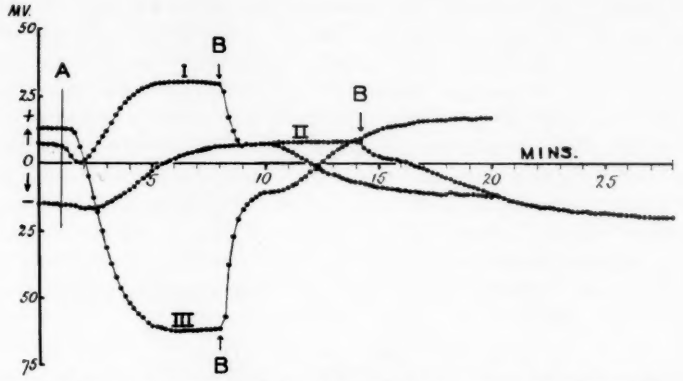

FIG. 7. Change of potential in basal *Elodea* leaf; notation as in figure 6.

sharp drop immediately, then a slowing down of the rate of decrease of potential, causing a flattening of the curve which may sometimes even take the form of a slight rise; this is then followed by a further rapid fall to approximately the original level of the potential between apex and base. There is no "over-shooting," that is, the potential reaches its final level slowly, not passing by it and then returning to it. In about 20 per cent. of the cases the double character of the descending curve cannot be discerned, perhaps because it is too slight.

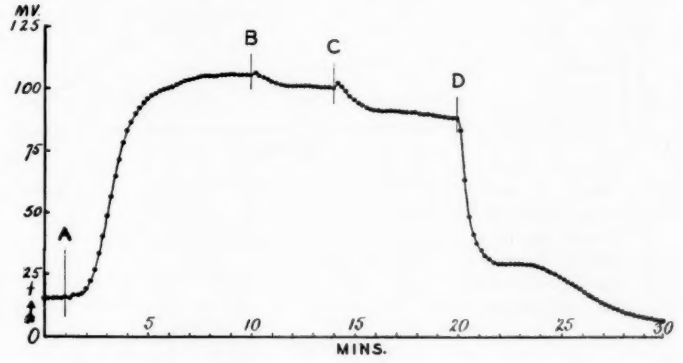

FIG. 8. Effect of decreasing intensity of illumination upon the potential of an apical *Elodea* leaf illuminated at apex:

A, illumination began; intensity 18,000 foot-candles;
B, intensity decreased to 9000 foot-candles;
C, intensity decreased to 1700 foot-candles;
D, illumination ended.

Preliminary experiments were also conducted upon the effect of varying the intensity of the incident light. This was done by means of a resistance inserted into the lighting circuit, capable of cutting down the intensity of the illumination of the spot upon the leaf from 18,000 to approximately 1700 foot-candles. It was found that when the intensity of the light was first applied at this maximum and then diminished, a corresponding diminution of potential was to be observed (fig. 8); but when the intensity of the light was first applied at 1700 foot-candles, the response observed was the maximum, further increase of the intensity failing to raise the potential (fig. 9). Evidently the intensity of the light is not the only limiting factor concerned here. Further work is being planned to determine this point fully.

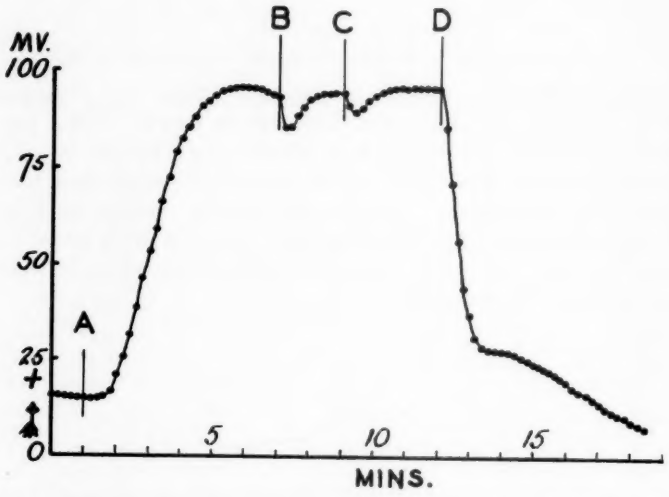

FIG. 9. Effect of increasing intensity of illumination upon the potential of an apical *Elodea* leaf illuminated at apex:

A, illumination began; intensity 1700 foot-candles;
B, intensity increased to 9000 foot-candles;
C, intensity increased to 18,000 foot-candles;
D, illumination ended.

Discussion

WALLER (**8**) reported potentials from many plants. The highest potential he observed amounted to only 15 millivolts under illumination. SHEARD (**4**) reported 0.3 volt in sunflower leaves under illumination; even this high potential, in relation to the area of leaf surface, is small in comparison with the potentials reported in the present paper for *Elodea*. The leaf of the

latter measures only 10–12 mm. in length and 3–5 mm. in width, yet potentials up to 100 millivolts have been observed under illumination between its apex and base. It should be evident, both from the size of the potentials observed and from their great constancy of behavior, that we are here concerned with something connected with the fundamental energy processes of the organism. It can hardly be believed that any expenditure of electrical energy by a plant in which the potential of a single leaf may vary by as much as 100 millivolts can be either insignificant or of secondary importance.

The relation of photosynthesis to these phenomena should prove of decided interest. The peculiarities of the curves found should throw further light on the question, particularly as to the chemical and physical processes involved, which give rise to them.

Transmission of the effect of illumination to non-illuminated regions is obvious. Illumination of a point on the leaf outside the region between the contacts has also been found to change the magnitudes of the E.M.F.s between the contacts. Figure 3 is particularly clear in showing such transmission. It should be observed that, since the decrement of E.M.F. along the leaf is practically a straight line, this means that the effect of illumination is felt not only at non-illuminated regions, but that the effect extends throughout the leaf, regardless of the distance from the point illuminated. This makes it very probable that the effect of light upon the potential of the leaf is not a direct photoelectric effect, but that it reacts first upon the chloroplasts, which in some way set up changes in the state of the leaf, giving rise in turn to the electric phenomena.

Summary

1. Apical and basal leaves of *Elodea* respond to illumination of the apex of the leaf by a large increase of the potential of the apex with respect to that of the base. The decrease in the magnitudes of the E.M.F.s along the leaf from apex to base in such a case is uniform.

2. Apical and basal leaves illuminated in the middle show a positive response, but to a less extent than when illuminated at the tip.

3. Apical and basal leaves illuminated at the base show a reversal of the electrical polarity found in the two preceding cases. The base becomes positive to the apex.

The writer wishes to express his deep appreciation of the material assistance rendered him in the present survey, both in the matter of apparatus and advice, by Dr. E. J. LUND.

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RELATION OF HYDROPHILIC COLLOIDS TO HARDINESS IN CABBAGE, BRUSSELS SPROUTS, AND ALFALFA PLANTS AS SHOWN BY THE DYE ADSORPTION TEST

STUART DUNN

(WITH THREE FIGURES)

Introduction

If we assume that hydrophilic colloids are largely responsible for hardiness in plants, then the amount of dye removed from solution by plant tissues suitably prepared will bear a direct relation to the degree of hardiness exhibited. In previous work by the writer (**1, 2**), in which anilin dyes and more particularly malachite green were used in a study of hardiness in the apple, somewhat conflicting results were obtained, traceable in part to the material and in part to the mode of sampling. Further experiments were thought desirable, and the present paper describes the results secured with cabbage, brussels sprouts, and alfalfa, which could be grown in pots in a controlled environment.

Materials and methods

Except in the case of alfalfa, where field-grown plants were used in some instances, all the plants used were grown in the greenhouse. For some of the hardening experiments, the potted plants were stored at 3° C. for various lengths of time in a lighted electric refrigerator, while other sets of plants for purposes of control were stored at room temperature in a specially constructed artificial light chamber and thus were grown under similar conditions except temperature.

Freshly macerated tissue was used in nearly all tests as it most closely resembles the colloidal conditions of the plant cell. The grinding was done by pressing the material against a rotary, motor-driven wire brush. Brussels sprouts were used in a nearly mature state, and their pith provided a perfectly uniform tissue obtained by paring away the outer parts.

After the tissue was ground, 1-gram samples of it were at once weighed into centrifuge tubes on a torsion balance sensitive to 5 mg. To each sample was added 25 cc. of the dilute dye solution (in the case of malachite green usually at a strength of 0.1 gm. per liter), and the tubes shaken vigorously for several minutes, centrifuged, and the supernatant liquid poured off into a test-tube for clearing. A considerable amount of the plant material remained in solution or suspension in the dye solution, even after centrifuging, which interfered somewhat with obtaining an accurate colorimetric reading. It was found that most of this could be coagulated by heating

in a boiling hot-water bath for three minutes. Tests on pure dye solution showed this treatment to have no effect on the color intensity of the dye. After cooling, the sample was filtered through an asbestos mat in a Gooch crucible. A portion of the solution was first run through and thrown away, to avoid the effect of adsorption of the dye by the asbestos. After filtration, the sample was tested for the amount of dye remaining in solution in a Leitz bicolorimeter, following the conventional procedure (**1**). In most cases it was possible to obtain a good match of colors without resorting to a second wedge containing a distilled-water extract of the plant tissue.

For check on the actual hardiness of different groups of plants, some of them were frozen at $-5°$ C. in a cold chamber in the cold storage plant of the Horticultural Department. In hardening off large plants such as mature brussels sprouts, which were too large to be put into the refrigerator, they were placed in another chamber of the cold storage plant at a temperature of 0° C. Since HARVEY (**3**) has shown that hardening is probably a cold shock response, and that exposure of plants to short periods of cold daily is very effective, in most cases the plants which were being hardened were placed in the cold chambers daily from 5 to 9 P. M.

Alfalfa plants presented some peculiar difficulties. Freezing tests showed that the tops were all killed in most treatments so that observations and tests had to be confined to the roots. Not being readily visible, like the tops, they must be removed from the soil and washed for accurate examination, which damages them for observation of injury at subsequent intervals. Their tough and woody nature renders freezing injury difficult to detect.

Experimentation

INVESTIGATIONS ON CABBAGE.—To gain comprehensive information as to the effect of different lengths of time of hardening on the colloid content of cabbage plants, a large number of plants were hardened for various periods from 1 day up to 12 days. HARVEY has shown that 5 days of hardening is sufficient to show marked differences between hardened and unhardened plants. Similar lots of plants were also submitted to a freezing test as a check on their cold resistance. The dye adsorption results are given in table I. The figures represent the percentage of dye left in solution after adsorption, and thus the lower values indicate the greater hardiness. Individual plants were ground separately, all parts above ground being used.

It will be seen that in general the average adsorption increases up to the 4- and 5-day periods, but beyond that there is somewhat of a decrease, especially in the group hardened for 12 days, which became rather unhealthy in appearance. In all cases the average adsorption was greater in the hardened plants than in the unhardened. It will be noted, however,

TABLE I

ADSORPTION OF MALACHITE GREEN BY MACERATED FRESH CABBAGE

TREATMENT	PERCENTAGE DYE REMAINING IN SOLUTION		
	MEAN*	MAXIMUM*	MINIMUM*
	per cent.	*per cent.*	*per cent.*
Hardened 1 day	32.4	46	21
Unhardened	40.4	59	31
Hardened 2 days	36.8	65	20
Hardened 3 days	33.1	55	23
Unhardened	39.3	57	25
Hardened 4 days	26.9	49	19
Hardened 5 days	29.8	45	20
Hardened 6 days	33.7	53	28
Unhardened	38.3	51	24
Hardened 8 days	37.4	50	24
Hardened 12 days	37.5	57	23

* From duplicate tests on 8 plants.

that the individual values for the different plants show great variability, from extremely low readings of around 20 up to high ones above 60. Each group has certain of these variations, even the unhardened lots. This naturally leads to the question of how the similar groups of plants behaved in simultaneous freezing tests. In most cases the results agreed in general with those of the dye adsorption tests in table I. Here also, however, wide

FIG. 1. Cabbage plants hardened for 3 days, then frozen for 24 hours at −5° C.

variation was found in the ability to resist freezing on the part of individual plants in all of the groups. Those affected most were completely wilted after removal for a short time from the freezing chamber, while others appeared almost unharmed. Various degrees of resistance on the part of some of these plants may be seen from the accompanying photographs. Figure 1 shows cabbage plants hardened for 3 days and then frozen at $-5°$ C. for 24 hours. Considerable variation may be seen, from one almost totally killed to one harmed only slightly. Even more variation is shown in the group in figure 2, which was hardened for 5 days before freezing. It is apparent that the group hardened for 3 days averaged less hardy than the group hardened for 5 days. Nevertheless the individuals of both groups show extreme variations, and this is probably the reason for some, if not most, of the variations found in the dye adsorption tests.

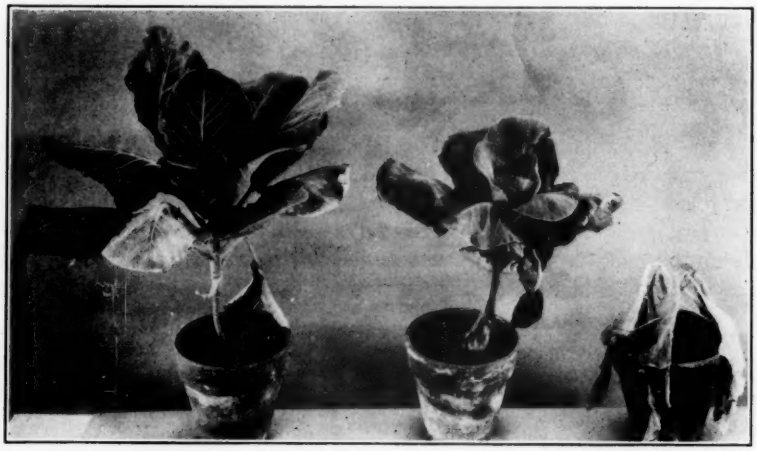

FIG. 2. Cabbage plants hardened for 5 days, then frozen for 24 hours at $-5°$ C.

It was observed also that different parts of the same plant, or different tissues, were differently affected, as can be noted from figures 1 and 2. In most cases the leaf blade was injured more than the petiole or remainder of the plant. This led to tests in which the leaf blades were not included, and the results of some of these are given in table II. In all cases there is, on the average, a greater adsorption on the part of the hardened plants, although there is still some variation by individual plants within the groups. It will be noted that in the groups of youngest plants, 6 weeks old, the difference in adsorption by hardened and unhardened plants is not so great as that of older plants. This is in accord with observations on the results of freezing tests. In general the older plants withstood freezing better than

TABLE II

ADSORPTION OF MALACHITE GREEN BY HARDENED AND UNHARDENED CABBAGE STEMS AND PETIOLES OF PLANTS VARYING IN AGE

AGE OF PLANTS	TREATMENT	PERCENTAGE DYE REMAINING IN SOLUTION		
		MEAN*	MAXIMUM*	MINIMUM*
		per cent.	*per cent.*	*per cent.*
Six weeks old	Hardened 4 days	54.8	58	52
	Unhardened	59.3	62	54
	Hardened 6 days	55.4	58	52
	Unhardened	59.6	65	54
Eight weeks old	Hardened 3 days	64.0	68	58
	Unhardened	68.4	76	61
	Hardened 4 days	60.9	66	53
	Unhardened	67.2	70	65
	Hardened 5 days	65.2	75	57
	Unhardened	69.6	77	64
Ten weeks old	Hardened 2 days	64.1	66	61
	Unhardened	76.0	79	73
	Hardened 3 days	73.7	80	65
	Unhardened	76.1	85	75
	Hardened 4 days	58.9	68	50
	Unhardened	75.0	78	68
	Hardened 5 days	54.0	57	50
	Unhardened	74.7	78	66

* From duplicate tests on 5 plants.

the younger ones. In the case of the petioles and stems of the cabbage plant, however, it is very difficult to determine by inspection the exact effect of freezing, since the larger amount of mechanical or supporting tissue renders the effect of the collapse of frozen cells less visible than that of leaf tissue. This greater proportion of supporting tissue may also be responsible for the smaller amount of adsorption where these parts of the plant were tested alone, than where the leaves were included (table I). This is even more strikingly noticeable in table III.

In view of the marked variations in the survival of individual plants within a group whose previous hardening periods or other previous treatment has been the same, and the possibility that this may also account for some of the variations encountered in the dye adsorption figures, an effort was made to test this by comparing plants unharmed by freezing with those injured. It should be noted here also that often there were marked differences in different parts of the same plant. The lower leaves frequently were killed and flabby and the upper leaves were not; or a part of a single leaf might be affected and another part not. The results given in table III are for groups of plants of somewhat different ages, but the

TABLE III

ADSORPTION OF MALACHITE GREEN BY HARDY AND NON-HARDY CABBAGE PLANTS DIFFERENTIATED BY FREEZING TEST

AGE OF PLANTS	TREATMENT AND CONDITION	PERCENTAGE DYE REMAINING IN SOLUTION					
		MEAN*		MAXIMUM*		MINIMUM*	
		LEAVES	STEMS	LEAVES	STEMS	LEAVES	STEMS
		per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
One month old	Hardy 3 days	30.0	59.0	39	61	24	55
	Non-hardy	33.5	67.7	35	69	31	66
	Hardy 7 days	32.5	63.0	36	66	25	60
	Non-hardy	38.0	74.2	40	77	35	71
Two months old	Hardy 4 days	35.5	57.4	38	65	32	52
	Non-hardy	44.4	65.9	49	68	40	62
	Hardy 6 days	33.3	57.1	36	60	31	53
	Non-hardy	43.6	64.1	46	68	40	59
Three months old	Hardy 2 days	38.4	.....	45	.....	31	.....
	Non-hardy	53.5	.....	60	.....	51	.....
	Hardy 5 days	31.3	.....	35	.....	27	.....
	Non-hardy	52.4	.....	58	.....	50	.....

* From duplicate tests on 5 plants.

outstanding fact is that the values show a uniformly greater adsorption by the hardy plant tissue than by the corresponding tissues of the non-hardy group. This is particularly true of the leaves. In the case of stem and petiole tissue, as has been noted before, it is often extremely difficult by examination to detect differences in these parts of the plant. This may explain partly some of the wider variations for these tissues given in the columns labeled stems. The results on the oldest plants, where only leaves were tested, confirm this more amply, and show that when it can be proved that a plant or plant part or tissue is hardy, then there will be a greater adsorption of dye by macerated samples of that tissue in comparison with non-hardy tissue.

An objection to this method of testing the plant tissue for adsorption after freezing the plant might be raised on the ground that the non-hardy plants which were so treated might have their colloidal properties altered by the effect of the freezing alone. An effort was made to determine this point by testing leaves of cabbage, from the same plant, cut in half lengthwise through the midrib. One group of halves was frozen and ground separately, while the unfrozen halves were also ground separately and tested for adsorption. Similar tests were made on whole cabbage plants halved lengthwise. In all cases the differences were slight and negligible.

This shows that the freezing is not a factor in producing the adsorptive differences noted in hardy and non-hardy plants.

INVESTIGATIONS ON BRUSSELS SPROUTS.—Large brussels sprouts plants were also tested, and the pith tissue of these plants was used because it is perfectly uniform, containing only one type of cell, with no fibrous elements not easily macerated. As in the cabbage plants, the average adsorption was greatest in those exposed to the hardening temperature for about 5 days. Here also, as in tests on cabbage plants, some attempt was made to select only those which definitely withstood freezing at – 5° C. for 12 hours or more as being hardy for dye adsorption tests. In the case of the pith tissue of these plants, however, it was much more difficult to detect differences in appearance of those injured. A water-soaked, darkened appearance was about all that could readily be seen. Frequently those plants having a hardy-appearing pith would have tender leaves and sometimes the reverse was true. Figure 3 gives an idea of the differences encountered after plants which had been treated similarly were frozen. These had

FIG. 3. Brussels sprouts hardened for 5 days, then frozen for 12 hours at – 5° C.

both been hardened for 5 days before being frozen for 12 hours at −5° C. Aside from these difficulties the results confirmed those upon cabbage, and for the sake of brevity the tabular data are omitted.

SPECIFIC ADSORPTION.—An attempt was made to narrow the field still further, from hydrophilic colloids in general, as indicated by malachite green, to some one chemical constituent or group of constituents. This was tried by the action of other dyes, some of which are more or less specific in their adsorption by certain groups of compounds. One of these was ruthenium red, which is a standard stain for pectic substances, and two others, Ponceau 3 R and light green S F, which are partially cytoplasmic stains and non-toxic. Two others were safranin and haematoxylin. The former stains lignin, cellulose, suberin, and protoplasm in histological preparations unless it is washed out, in which case it primarily stains lignin and suberin. Sometimes it is used as a precipitating agent for proteins. Haematoxylin stains cellulose walls only. Malachite green was also used as a check on the hardiness qualities.

Since these dyes were diluted for adsorption tests to about one-twentieth of the concentration used in histological work, microscopic studies were made with them at these dilutions, using cross-sections of young cabbage petiole and fresh *Elodea* leaves. The observations indicated that with safranin there was uniform penetration of most cells and accumulation in the vascular or heavily lignified cells and in the outer cutinized epidermis. Haematoxylin accumulated mainly in walls of parenchyma and moderately in the bast fibers and collenchyma of the cabbage. Light green S F and Ponceau 3 R penetrated the tissue fairly uniformly but plasmolyzed the cells slightly. No part of the protoplast was penetrated and there was no particular difference in color in any tissue. Apparently scarcely any of the dye was removed from solution. Malachite green penetrated the protoplasts of many cells of *Elodea* fairly uniformly, especially the chloroplasts and other plastids, but was not noticeable in the walls. In cabbage sections there was a somewhat noticeable accumulation in the cells rich in protoplasm near the outer edge, and in the vascular tissue.

A large number of cabbage and brussels sprouts plants were passed through a freezing test, and an outstanding plant showing marked hardiness and similarly one which was non-hardy were chosen from each. Each of the dyes was tested for adsorption on several samples from the same plant, using the usual colorimetric technique. It was found that with safranin there was a greater adsorption by the non-hardy samples. This would indicate that perhaps in the hardening process there is a conversion of lignin or allied materials and proteins into something else, perhaps some protoplasmic or cell sap constituents. With light green S F there was very slight adsorption, especially in the brussels sprouts, and with Ponceau 3 R

none at all. This agrees well with the observation on microscope sections already noted; and since there was the usual greater adsorption of malachite green by the hardy tissue, this would indicate that some of the protoplasmic constituents are probably primarily responsible for hardiness. Haematoxylin gave slight differences, indicating that the cellulose wall constituents are of slight importance in hardiness. A somewhat greater adsorption of ruthenium red by the hardy tissue, especially in brussels sprouts, shows that pectic substances may have a part in the colloidal protection against low temperature.

INVESTIGATIONS ON ALFALFA.—Seed of the same two varieties of alfalfa as those used by STEINMETZ (**4**) in his investigations was obtained from the same source. They were Kansas Common, a rather tender variety, and Grimm, a hardy variety. The seed was planted in outdoor plots in the spring. During the following winter and spring, samples of the roots were dug up in February, March, and April and tested for adsorption in the macerated fresh condition, and also dried and ground to a fine powder. It was found that the fresh samples of February and April gave tests correlating with the hardiness of the two varieties, while the March samples did not. The dried samples showed very little correlation. Even when the percentage of dry weight was taken into consideration, the differences were not sufficient to make that a factor. Presumably the heating in some way alters the colloidal substances of the tissue, and tests on fresh tissue may be regarded as more indicative of hardiness.

Other tests were run on hardened and unhardened potted plants. Efforts were first turned toward preliminary tests on the killing point of roots by freezing, using the water-soaked appearance and exudation of sap from a cut end mentioned by STEINMETZ as criteria of injury. All plants were old, with large roots, having been grown in large pots in the greenhouse for a long time. The Grimm and Kansas Common were used, and also a variety called Cossack, from Texas, supposedly tender. The time for killing roots removed from soil varied between 1 hour and 2.5 hours at $-4°$ C.

This work was attended with the difficulties mentioned under Methods, but in several instances differences were noticeable a short time after thawing, and results on a few of the many adsorption tests run are given in table IV. The first two groups, those unhardened and those hardened 11 days, show a greater adsorption by the hardy tissue, as anticipated from previous work. The other 9-day plants show conflicting results.

In a few other tests, from time to time, discrepancies of this nature were encountered, which led to some doubt as to the advisability of placing very great confidence in the water-soaked appearance of the root and other similar criteria as evidence of injury. Furthermore, WEIMER (**5**) has

TABLE IV

ADSORPTION OF MALACHITE GREEN BY MACERATED GRIMM ALFALFA ROOTS; INJURY DETERMINED BY INSPECTION SHORTLY AFTER FREEZING

TREATMENT	APPEARANCE	PERCENTAGE DYE REMAINING IN SOLUTION		
		MEAN*	MAXIMUM*	MINIMUM*
		per cent.	*per cent.*	*per cent.*
Unhardened	Not injured	19.6	24	18
	Injured	26.2	28	25
Hardened 11 days	Not injured	20.6	21	20
	Injured	36.2	47	29
Hardened 9 days	Not injured	31.7	38	26
	Injured	24.0	25	23

* From 5 samples.

shown that different regions or tissues, or even separate groups of cells, in the same alfalfa root may show differences in survival in freezing tests. This led to final attempts to determine more accurately the relation between dye adsorption and frost survival, by freezing hardened and unhardened roots and then placing them in wet sand to allow those surviving to bud out. Those showing differences were then selected for dye tests. One group of all three varieties was tested in this way after hardening for 8 days, and after a few days the hardened plants in most instances budded out more vigorously and a few of the unhardened ones were entirely killed. The differences in the Texas Cossack were very slight, and this is also true of the dye test on this variety. Results appear in table V.

TABLE V

ADSORPTION OF MALACHITE GREEN BY MACERATED ALFALFA ROOTS; INJURY AFTER FREEZING DETERMINED BY BUDDING OF ROOTS IN MOIST SAND

VARIETY	CONDITION	PERCENTAGE DYE REMAINING IN SOLUTION		
		MEAN*	MAXIMUM*	MINIMUM*
		per cent.	*per cent.*	*per cent.*
Grimm	Hardy	26.7	30	23
	Non-hardy	48.0	52	41
Kansas Common	Hardy	16.7	19	14
	Non-hardy	32.5	40	24
Texas Cossack	Hardy	23.5	30	19
	Non-hardy	18.5	23	16

* From 4 samples.

In this case also, it seems evident that hardiness, as demonstrated by survival of or resistance to freezing, is correlated with dye adsorption and therefore with hydrophilic colloid content; although even here it is exceedingly difficult to be sure of actual differences in survival.

As mentioned before, previous work on apple-twig tissue showed wide variations and disagreements in hardiness and dye tests. In the light of the results presented here, most of the seeming lack of correlation there was probably due to a lack of definite and accurate knowledge of the actual hardiness, not only of the individual tree but of the individual twigs used as samples for the dye adsorption tests. It is true that parallel freezing tests were made at the same time as the dye tests, but they were on other twigs, and from the preceding evidence it seems probable that at least some of the differences were due to this.

Another factor which may be of importance in this connection is that in ligneous plants of this type there is present in the twigs, and consequently in the samples tested, a large amount of tissue extraneous or foreign to the tissues actually involved in hardiness. That is, even though the colloid content of the living active cells concerned in the hardiness and vigor of the tree may be the main factor in hardiness, the percentage of these cells is so small as compared with other kinds of tissue that their effect is masked. This factor has already been mentioned in connection with the relation between the fibrous elements and visibility of killing effects in cabbage petioles and stems and alfalfa roots.

Summary

1. Previous investigations using the adsorption of the dye, malachite green, as a measure of the content of hydrophilic colloids in relation to hardiness in apple-twig tissue had been somewhat conflicting. Investigations were made on small succulent potted plants because their environment and growth conditions could be more readily controlled.

2. Dye adsorption tests were performed on cabbage, brussels sprouts, and alfalfa plants macerated in the fresh condition, and on dried tissue, to determine colloid content and to determine its relation to hardiness as shown by freezing tests on plants by artificial refrigeration.

3. Random tests on a large number of cabbage plants hardened for varying lengths of time showed that, on the average, hardiness as shown by dye adsorption increased over the unhardened plants up to 5 days' hardening. There were, however, marked extremes in values in both hardened and unhardened individuals. This, in general, agreed with results on parallel freezing tests where also extremes were met.

4. Tests on cabbage and brussels sprouts, in which actual survival ability or frost resistance of the individual plants was first tested by freezing,

showed that where this could be accurately determined, usually there was a greater amount of dye adsorbed from solution by such tissue, and the hydrophilic colloid content thus measured is probably in considerable degree responsible for hardiness.

5. Efforts were made to narrow the field from hydrophilic colloids in general to some specific group of colloids. Adsorption tests by different dyes which vary somewhat in their adsorptive action showed that: (1) pectic substances may be partially responsible for cold resistance; (2) lignin and allied substances and perhaps proteins are apparently converted into other compounds in the hardening process; and (3) protoplasmic constituents rather than those of the cell wall are probably of primary importance in hardiness of the plant cell to cold.

6. Work on alfalfa roots showed that here, too, dye adsorption was greater in the hardier plant, although results of freezing tests are more difficult to interpret because of the tough, woody nature of the tissue.

7. It is suggested that the conflicting results obtained in some of the previous work on apple-twig tissue may have been due partly to the lack of accurate knowledge of the cold resistance of the twigs actually tested, and partly to the fact that the vital tissue concerned in hardiness is a very small part of the total bulk of the twig, so that the colloidal effect of such tissue would be masked by other inactive parts.

The writer wishes to express his sincere appreciation to Dr. R. B. Harvey of the University of Minnesota and to Dr. O. Butler of the University of New Hampshire, whose kindly criticisms and advice have aided these investigations.

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STIMULATIVE EFFECTS OF X-RAYS ON PLANT GROWTH[1]

CHARLES A. SHULL AND JOHN W. MITCHELL

(WITH FOUR FIGURES)

Introduction

During the period since the discovery of x-rays by RÖNTGEN in 1895, a vast amount of work has been done in which these radiations have been used for clinical diagnosis and therapy. The practical applications of x-rays in medicine and surgery make it necessary to know the effect which x-rays produce upon the living organism. Many investigators have suggested on the basis of general observations that small doses of x-rays may stimulate cellular activity and growth, but convincing proof of such action has been wanting. In more recent years such claims have been discounted in favor of the belief that x-rays are always more or less destructive in action, and tend to retard growth.

It is not the purpose of this preliminary report to survey the literature dealing with the effects of x-ray treatments upon plants. It has been found that every part of the plant body can be profoundly modified by appropriate treatments. Cytological and histological examination of treated cells and tissues reveals striking changes in the organization of the protoplasm and of organs derived from the treated meristems. Most frequently the results described are of a destructive nature. The protoplasm is partially disorganized; chromosomes are vacuolated or fragmented; the cell division mechanism functions imperfectly, showing unequal distribution of chromosomes, non-disjunctions, translocation of pieces of chromosomes from one to some other non-homologous chromosome, etc. Gene changes may be produced, often injurious in character, with resulting lethal effects and tendency to sterility. The results obtained by MCKAY and GOODSPEED (**5**) on cotton are typical. Many mutations have been induced in maize and barley (**7, 8**), and tobacco (**1**), but it has been questioned whether there are any progressive evolutionary changes induced by x-ray treatments.

All vegetative parts are subject to injury by x-rays. Root tips may become bulbous and swollen, with tumor-like enlargements in which giant cells may occur. Stems become fasciated under strong treatments. Leaves are injured readily; they become asymmetric and crumpled in appearance, develop deep sinuosities, and often show irregular development of chlorophyll. The sunflower shows these injuries in typical fashion, the leaves becoming pocked and marked as though they were suffering from a mosaic

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

disease. Even the flowers of plants rayed in seed or seedling stages may show fasciation or various teratological modifications. Some of these have been described for the sunflower and tomato by JOHNSON (**2, 3**).

On the other hand, one can find a dozen or more claims in the literature that x-rays in small doses are stimulative. In some cases increased yields have been claimed for crops grown from x-rayed material. Such claims have been reinvestigated in some cases, and the stimulative effects denied. JOHNSON (**4**), for instance, has not been able to substantiate such claims made for the potato. However, some increase of yield has been reported for x-rayed potatoes at the New Jersey Agricultural Experiment Station. PATTERSON and MULLER (**6**) have found that induced point mutations in *Drosophila* (presumably caused by chemical changes in the genes) may cause increased vigor in some cases. They argue in favor of the possibility of progressive x-ray mutations with endless eventual potentialities.

As a result of our experiences with the use of x-rays on plants it is believed that stimulative effects may be consistently obtained if appropriate conditions are employed. Possibly these stimulative phenomena have not been regularly detected in the past because the intensity of the radiations have been too great, or possibly because the x-ray beam contained too large a proportion of long wave-length radiation. Deleterious effects are consistently obtained in our work when unfiltered radiations are used, and we believe that these harmful effects mask the stimulation that occurs when the beam is properly filtered. Filtration of the radiation, of course, affects the wave-length constitution of an x-ray beam profoundly. It not only reduces the intensity of each wave length throughout the x-ray spectrum, but also changes the relative proportion of the energy supplied by each wave length throughout the spectrum. The shorter radiations suffer much less absorption than the longer radiations; and for practical purposes the longest x-rays are so strongly absorbed by aluminum or copper filters that filtration through such metal plates practically removes them from the beam.

Since filtration affects both the intensity and relative composition of the beam, and since we have not yet differentiated these effects in our work, we are not in position to discuss the nature of the x-ray action. Until further experiments are done we cannot say whether the stimulating effects that are obtained when the beam is filtered are due to the fact that harmful long wave-length rays are removed, or whether they simply indicate that stimulation follows low intensity irradiation, regardless of wave length, and is masked by injury if the intensity is greater, regardless of wave length.

Believing that the dosages in common use for treatment of plants were much too large, we have used very small doses. The intensity of the radia-

tions used is expressed in Röntgen units measured with a Wulf ionometer,[2] the measurements being taken in air without the effect of back-scattering of the beam by solid material. We are indebted to Dr. Paul C. Hodges, Röntgenologist of the University of Chicago, for the calibration of our instrument, and for many helpful suggestions.

In these preliminary experiments we are using about 100 pk. KV., 5 ma., 1-mm. aluminum screen. Under these conditions the instrument delivers about 38 r-units per minute at a point 30 cm. from the target, the distance used in these experiments. Our experimental material is exposed on cellucotton pads in glass dishes resting on a lead-covered table. It undoubtedly received slightly higher doses than were computed in air because of a slight amount of back-scattering of the radiations. But the computation of the dose in air is a standard method of measuring the dosage. In some instances our best results have been obtained with 1 minute or less, a total of 30–40 r-units. In most cases maximum stimulation has been obtained with not more than 2 or 3 minutes; and with 4 or 5 minutes the effect is already one of retardation of growth.

It is evident at once that investigators who have been using from one to ten erythema doses as light doses, are using extremely heavy doses. The erythema dose is a rather rough unit of measurement, and may be defined as that dose of x-rays that just fails to produce a detectable change in the normal human skin. It is at best a vague designation, but is still much used. It seems much better to adopt the more accurate r-unit. It is generally accepted that the physical equivalent of the erythema dose is approximately 600 r. The Holzknecht is also used in expressing x-ray doses, and this is approximately 120 r.

The optimum dosage for different kinds of plants is probably specific, and must be determined by experiment for each species and varietal strain. A number of common plants seem to respond best to dosages between 30 and 120 r.

Methods

In order to make it possible to repeat our procedure, the details of preparation of the seeds for treatment are given. Seeds of such plants as corn, wheat, oats, and sunflower have been used. They are placed for 24 hours in a moist chamber upon a layer of cellucotton saturated with distilled water, and kept at a temperature of about 22° C. The seeds are used without sterilization, and lie in contact with the wet substrate on one side, and in contact with moist atmosphere on the other side. They are not

[2] Small-chamber instruments of this sort are intended primarily for use with higher voltages and are somewhat inaccurate at lower voltages. Eventually the calibration will be checked with large-chamber instruments that are relatively insensitive to voltage change.

submerged during the period of preliminary imbibition and germination. At the end of 24 hours the seeds of all four species show incipient germination. The radicles protrude through the pericarps and enable one to know that the seeds are alive. At this stage the material for treatment and for controls is selected. Twenty or more seeds as nearly at the same stage of germination as possible (estimated by equal length of protruding radicles) are chosen and divided into two lots. One lot is left untreated, the other is placed upon fresh saturated cellucotton and treated at once for 1–5 minutes. Optimum effects are often obtained with 1, 2, or 3 minutes of treatment, according to species. Sunflower seems best at 3 minutes, corn possibly at 2 minutes, and some varieties of wheat at 2 minutes. In some cases wheat gives good results at 30 to 45 seconds or 1 minute. As soon as the raying is completed, controls and treated seeds are both planted in the same type of soil, or in sand culture, or on fresh saturated cellucotton in a moist chamber, depending upon the nature of the experiment. In the case of respiration experiments, controls and treated seeds are placed on a wet substrate in the respirometer immediately after treatment. During treatment the glass covers of the moist chambers or petri dishes are removed so that the only screen is the metallic aluminum screen. In the case of sunflower seeds the pericarps of the fruits are removed before treatment. They are also removed from the controls before planting. We have tried to avoid any differences except that of the treatment itself. Selection of seeds is practiced only to obtain material of uniform physiological activity for the controls and treatments.

Results

Wheat

The first tests with Marquis spring wheat indicated that it is sensitive to small doses of x-rays. The treated plants were decidedly more vigorous than the controls when the period of exposure was from 45 seconds to 1 or 2 minutes. By the time the plants were several weeks old (in soil culture), the treated individuals were taller and of ranker growth. The greatest difference was in the degree of tillering. The untreated plants showed 50 per cent. with one tiller each, while the treated plants showed 100 per cent. with two tillers each. Figure 1 shows the general appearance of the plants on September 17, after several weeks of growth.

Tests with Minhardi and Trumbull wheat gave us the impression at the time that the hardier variety (Minhardi) was less easily influenced by x-rays. The Minhardi wheat in the first tests seemed to show little stimulation, while Trumbull, a moderately hardy variety, showed plainly that its early development was hastened by treatment, but not so much as the Marquis spring wheat. At the present time we are not certain as to the order of

these varieties with reference to degree of stimulation.[3] It is possible that varieties more stable toward cold treatments may also be more stable toward x-ray action. We believe the dosage is specific for each variety, and that a longer treatment may possibly be required by the hardier varieties to produce a given amount of stimulation.

Corn

The most interesting results were obtained with Madison Yellow Dent corn. It was noted that grains which had been treated emerged from the soil more rapidly. On September 22, seeds which had been imbibing water for 24 hours were treated 1–5 minutes, one series screened by aluminum, another treated without metallic screen. A third series, untreated, served as controls. Five days later the seeds treated through the screen showed 84 per cent. of emergence; the unscreened treated seeds showed 72 per cent.;

Fig. 1. Influence of x-rays on growth of wheat: Pot at left rayed 1 minute; at right, 45 seconds. Controls in middle pot. For other conditions see text.

and of the controls only 60 per cent. had emerged. Treated seeds kept in petri dishes always showed a more rapid elongation of coleoptiles than untreated seeds. We have removed such coleoptiles from the seeds at the end of three days and determined the fresh and dry weight of the coleoptiles. Treated seeds showed from 5 to 26 per cent. greater fresh weight than the controls, and from 3 to 16 per cent. greater dry weight. This suggests the possibility that there is a more rapid utilization of the endosperm reserves in seeds that have been treated.

When the treated corn seeds were grown for a few weeks, some very important differences were noted. Figure 2 shows corn grown from seeds treated 1–5 minutes under an aluminum screen. While the growth differences are visible, and somewhat irregular, the main differences in this set are not visible to the eye in the photograph. The plants treated for

[3] Work on these varieties of wheat is being continued by Miss Bessie Zabelin.

TABLE I

INFLUENCE OF X-RAYS ON GROWTH OF CORN

TREATMENT	STEM DIAM.		FRESH WT. ROOTS		DRY WT. ROOTS		FRESH WT. TOPS		DRY WT. TOPS		CHLOROPHYLL* FRESH WT.		CHLOROPHYLL* DRY WT.		
	mm.	%	*gm.*	%	*gm.*	%	*gm.*	%	*gm.*	%	%	*mg./cm.²*	*mg.*	%	%
Control ...	5.63	100.0	54.7	100.0	5.11	100.0	49.7	100.0	4.655	100.0	0.1006	0.0125	5.03	0.874	100.0
1 min.	5.85	103.9	58.8	107.5	4.76	93.1	65.4	131.6	6.03	129.5	0.1341	0.0163	6.70	1.22	139.6
2 min.	6.73	119.5	54.7	100.0	4.79	93.7	86.1	173.2	7.67	164.7	0.1688	0.0219	8.44	1.43	163.6
3 min.	6.15	109.2	54.2	99.0	3.69	72.2	63.9	128.5	5.85	125.6	0.1517	0.0201	7.58	1.34	153.3
4 min.	6.47	114.9	60.6	110.8	4.80	93.9	86.6	174.2	7.30	156.8	0.1573	0.0205	7.86	1.57	179.6
5 min.	5.45	95.2	62.2	113.7	5.66	110.7	65.1	130.9	6.44	138.3	0.1407	0.0178	7.03	1.21	138.4

* Chlorophyll determinations according to GUTHRIE, made by Mr. G. B. ULVIN.

short periods (1–3 minutes) had thicker stems than the controls, or those treated 5 minutes. The treated plants looked and felt slightly more succulent, and were darker green in color. The fresh green weight of the tops was obviously greater in the treated plants than in the controls. Without detailed discussion we present in table I such differences as were measured. The chlorophyll differences need further investigation, as this darker green color was not noticed in the oats, wheat, and sunflowers.

The irregular growth of the 3-minute plants in figure 2 may have been caused by a defect in the instrument which was not discovered and corrected until after several lots of seeds had been treated. In table I the most important data are those on dry weight increase (column 11) and those on chlorophyll increase (column 16).

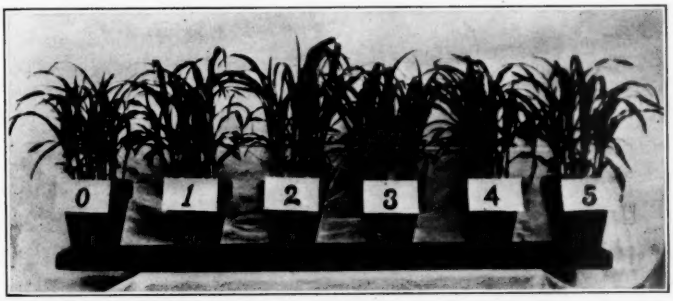

FIG. 2. X-rays and the growth of corn. Control at the left. Time of treatment in minutes indicated on the pots. For other conditions see text.

In table II are presented data on the moisture content of the roots and stems. While the differences are small, they affect roots and tops alike.

TABLE II

WATER CONTENT OF X-RAYED CORN PLANTS

TREATMENT	ROOTS		TOPS	
	DRY WEIGHT	WATER	DRY WEIGHT	WATER
	per cent.	*per cent.*	*per cent.*	*per cent.*
Control	9.34	90.66	8.58	91.42
1 min.	8.09	91.91	8.32	91.57
2 min.	8.75	91.25	8.03	91.97
3 min.	6.78	93.22	8.25	91.75
4 min.	7.92	92.08	7.87	92.13
5 min.	9.14	90.86	9.06	90.94

With light doses, the dry weight percentage decreases and the water content increases. Even these small differences are large enough so that the practiced eye and touch can detect the greater succulence of the plants from seeds treated for 1–3 minutes.

Oats

Only one experiment has been performed with oats. The seeds were from a laboratory sample without name. The increased growth of treated seeds was irregular, as in the case of corn, but plainly visible in all of the treated material. Figure 3 shows the results with plants from seeds

Fig. 3. X-rays and the growth of oats. Control at the left. Time of treatment in minutes indicated on pots. For other conditions see text.

rayed through a 1-mm. aluminum screen at 30 cm. for the periods of time marked on the pots. A defective contact in the machine is believed to have been responsible for the irregular behavior at 2, 3, and 4 minutes, but even these showed increased growth in height and thicker culms than the controls.

Sunflower

The sunflowers were treated after the x-ray machine had been repaired. In figure 4 the controls and treated plants show an excellent curve of height growth. In the photograph the 2-minute and 4-minute plants were omitted. They were perfectly intermediate between 1 and 3 minutes, and 3 and 5 minutes respectively. The 10-minute plants were rayed without the screen. These unscreened plants show the symptoms of burning described by Johnson (**2**). The leaves are asymmetrical, distorted, pocked as if they had mosaic, and the plants are greatly stunted. The screened plants show none of these ill effects; leaves are normal in every way, and growth more rapid. The group of plants rayed 3 minutes blossomed first, indicating a slight shortening of life history by the treatment.

Some attention has been given to the carbohydrate metabolism and respiration of treated seeds. Under the methods we are using, a slightly more rapid liberation of sugar is detectable from the reserves of corn, and a

slightly more rapid respiration of rayed seedlings. The increases are not very striking, and we feel that the data are too meager to be published at present. It seems hardly possible that the increased rate of emergence of seedlings, increased rate of growth, etc., could take place without some increase in respiration rate. This may be controlled in part by the concentration of sugar in the protoplasmic environment. The first tests on diastatic activity, however, showed distinct depression of the enzyme by x-ray treatment. Much more extensive tests must be made on sugar concentration, respiration, and enzyme activity with material more favorable than corn for this purpose.

Conclusion

From the results obtained in these preliminary experiments it is concluded that if the x-rays are properly filtered to decrease the intensity of the beam, or to decrease the proportion of the longer radiations, and if the quantity of energy used is adjusted to the specific requirements of the

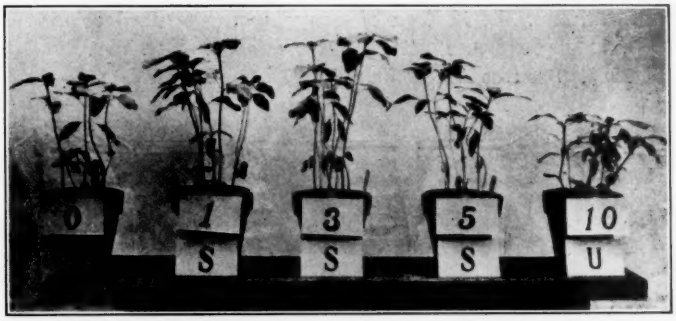

FIG. 4. X-rays and growth of sunflowers. Control at left. Time of treatment in minutes indicated on pots. Plants at right unscreened. For other conditions see text.

plants by control of the duration of radiation, and of the voltage and amperage used, plants can be stimulated to show increased growth rates.

Summary

1. A few preliminary experiments are described which indicate that under appropriate conditions of treatment, x-rays produce stimulative effects upon plant growth. Wheat, corn, oats, and sunflower seedlings have been used.

2. The seeds were treated in an early stage of germination after soaking for 24 hours in a closed moist chamber on a substrate of cellucotton saturated with water. The seeds are not submerged during soaking, but are wet on one side, and in contact with air.

3. The conditions which we believe necessary for such stimulative action are: the use of metallic screens, high voltage and low amperage, and brief exposures. The total dosage for stimulation does not much exceed 100 r-units. Even with the 1-mm. aluminum screen sunflowers given 150–200 r-units were overtreated. Optimum growth occurred with about 115 r-units (3 minutes).

4. There is some evidence of increased sugar content and increased respiration of treated seedlings.

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DECREASING HARDINESS OF WINTER WHEAT IN RELATION TO PHOTOSYNTHESIS, DEFOLIATION, AND WINTER INJURY[1]

S. T. DEXTER[2]

(WITH THREE FIGURES)

The hardening of plants with the approach of the winter season has been the subject of numerous studies; the decreasing hardiness of plants with the approach of spring has received little attention. DEXTER (**1, 3**) and DEXTER, TOTTINGHAM, and GRABER (**4, 5**) have made use of methods of studying hardiness which involved the principle of changed permeability and electrical conductivity of tissues injured by freezing. Plants were hardened under various controlled conditions and it was found that they hardened poorly or not at all in the dark, when low in organic reserves; plants in the same condition hardened well if given opportunity for photosynthesis at low temperatures. If the conditions during hardening were such that material vegetative extension occurred, hardening was in general lessened thereby. If, during part of the day and especially at night, the respiration rate of the plants was raised by elevating the temperature, hardening was inhibited (see also PELTIER and TYSDAL **6**). In general the development of hardiness was favored by environmental conditions which would seem to tend toward the accumulation or conservation of the organic food supply.

This paper deals with the application of these general principles to the problem of the return of the plant to the less cold-resistant condition. There appears to be little if any description of work done in regard to the rapidity of development of this condition, or the factors influencing it.

Experiment 1

Samples of two varieties of winter wheat were brought into the greenhouse at monthly intervals throughout the winter. The varieties, Minhardi (very hardy) and Wisconsin Ped. no. 2 (a hardy Turkey selection), were seeded in 12-inch pots and placed in soil out-of-doors, on September 23, 1931. The soil was of good fertility. The rather loose seed bed appeared to favor a rapid and somewhat more vegetative growth than was found in plants seeded in adjacent unworked ground. The plants in the pots were somewhat less hardy than those in the firm soil, but they appeared to be in no way unusual for winter wheat.

The weather during the winter was generally mild, although the temperature frequently went below freezing. Figure 1 shows the temperature

[1] Contribution from the Hull Botanical Laboratory, University of Chicago.

[2] National Research Council Fellow in the Biological Sciences.

record for the period. Up to December 18, when the first samples were taken, there had been no weather cold enough to kill the leaves of the wheat, and both varieties were in excellent condition, with a large photosynthetic area. During the interval from December 18 to January 18, no weather conditions occurred which would kill the wheat leaves, and on the latter date the foliage was still as green as on December 18. During January 29 to 31, however, the temperature dropped sharply, with no snow cover, and the plants were almost completely defoliated by the cold. A period of warm weather followed and new leaves promptly developed. These leaves, about 1 inch in length, were on the plants at the time of the February 18 determinations. In the greenhouse these plants (February 18 samples) continued to grow with rapid foliar elongation, whereas on the previous dates little or no vegetative extension could be seen. In early March the temperature again became sufficiently low to kill the leaves by freezing, although there was some snow cover accompanying the low temperature. This cold

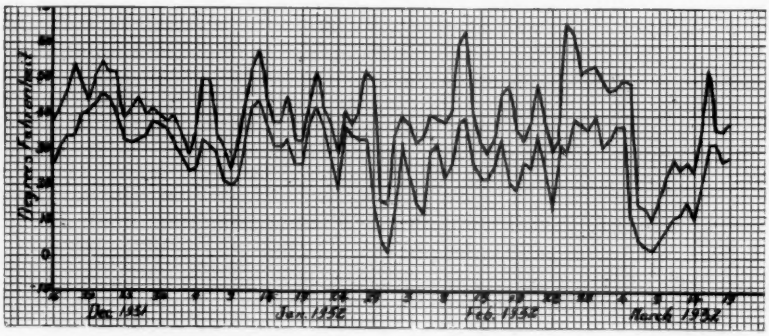


FIG. 1. Daily maximum and minimum temperatures, in ° F., at Chicago, Illinois, for the dates indicated.

period was of considerable duration and the ground became deeply frozen for the only time during the winter. The new growth (since February 1) was completely killed and the Wisconsin Ped. no. 2 plants were obviously severely injured by the cold. In the greenhouse, following March 17, renewal of leaves on Minhardi plants was rapid; only a few of the Wisconsin Ped. no. 2 plants recovered.

To study the condition of these plants throughout the winter, the following scheme was used. On December 18, January 18, February 18, and March 17, one pot of each variety was brought from the field into the greenhouse, where they were exposed to the daylight prevailing on the respective dates, at a temperature held at about 62° F. The hardiness of the plants was determined when they were brought in, again after two days in the

greenhouse, and then after seven days in the greenhouse, by the freezing-exosmosis test used in previous experiments (**2**).

Some difficulty was anticipated in obtaining relatively constant conditions during the period allowed for decreasing hardiness. The weather, however, happened to be favorable in that regard. There were no ice crystals in the pots on December 18 when they were brought in, although the soil had been very recently frozen. The same was true on January 18. On the night of February 17 the temperature dropped, freezing the soil to a depth of 1 to 2 inches. During the cold weather early in March, the soil froze to a depth of about 12 inches, but a day or two of warm weather preceding March 17 had thawed the upper 3 or 4 inches. Little difficulty was experienced in keeping the greenhouse at the approximate temperature of 62° F.

The samples were prepared as usual: duplicate 2.5-gm. samples of crowns were frozen in tubes at – 15° C. for 2 hours in an alcohol-slush bath, thawed for 30 minutes at 2° C., and 25 cc. of water at 2° C. were then added to each tube. Exosmosis continued for 16 hours at 2° C., when the conductivity readings were made. (In some cases, 1-gm. samples were used with 10 cc. of water, with other procedures the same. This did not appear to alter the values.)

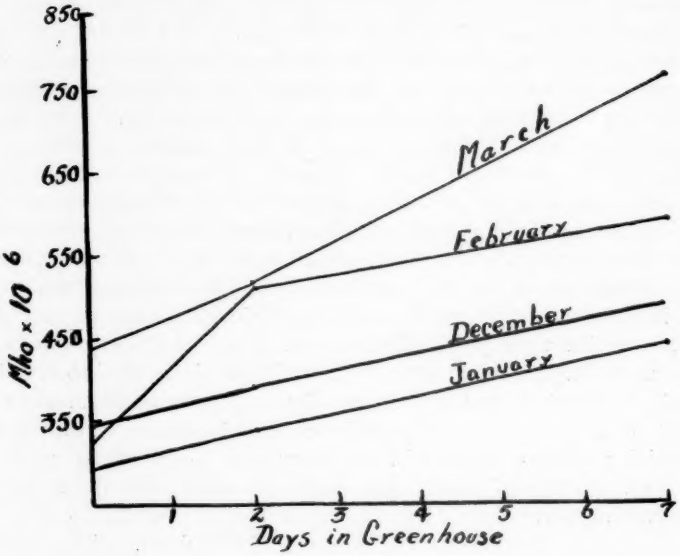
Table I shows the results secured on the different dates. Figures 2 and 3 show the data in graphic form for the two varieties.

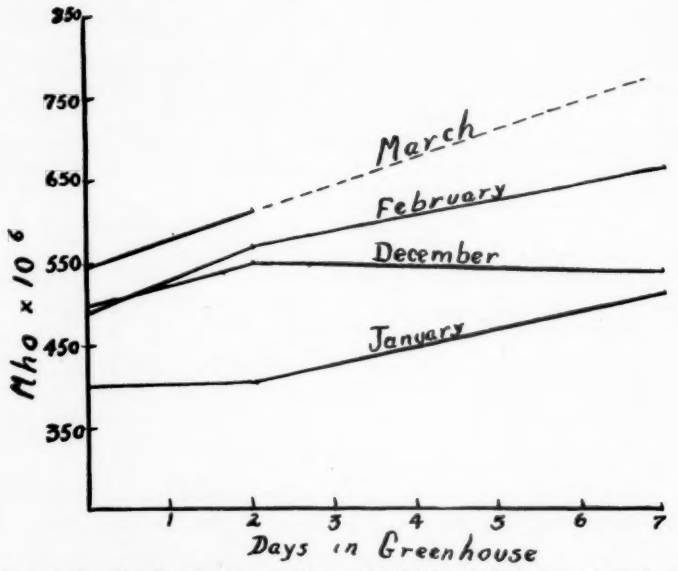
TABLE I

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 2° C.) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS OF CROWNS OF WINTER WHEAT FROZEN FOR 2 HOURS AT – 15° C. INTERVAL OF 16 HOURS ALLOWED FOR EXOSMOSIS AT 2° C.

VARIETY	DATE BROUGHT IN	DAYS IN GREENHOUSE AT 62° F.		
		0	2	7
Minhardi Wis. Ped. no. 2	December 18	342 496	391 548	490 539
	January 18	294 392	342 400	443 515
	February 18	329 493	511 562	592 658
	March 17	439 548	516 605	767 Dead, no sample

On every date and at every stage of decreasing hardiness, Minhardi was found to be hardier than Wisconsin Ped. no. 2. On December 18 the varieties were somewhat hardy, and their hardiness decreased rather slowly in the greenhouse, being far from complete at the end of one week at 62° F.





FIGS. 2, 3. Graphs showing specific conductivities ($\times 10^6$) in reciprocal ohms of extracts from two varieties of winter wheat. Fig. 2 (above), Minhardi; fig. 3 (below), Wisconsin Ped. no. 2.

On January 18 both varieties were distinctly hardier than in December, and decreased in hardiness still more slowly. These plants had had abundant opportunity for photosynthesis during the intervening month, had suffered no defoliation nor winter injury, and had made no visible vegetative extension. It seems highly probable that photosynthesis had more than balanced respiration, since there was little or no snow, the plants only occasionally frozen, and soil water available.

The sharp frost at the end of January introduced another feature. Winter injury at this time was considerable, although there was no evidence that any plants of either variety were killed outright. The warm weather following stimulated prompt elongation of the inner leaves, even though the soil was scarcely thawed. This growth no doubt used a part of the organic reserves of the plants and some decrease in hardiness was found on February 18, as the conductivity values show. In the greenhouse following this date, vegetative growth continued rapidly, together with a pronounced decrease in hardiness. In the same way the cold weather during the first part of March killed the extensive new growth made since the end of January. Not only that, but it killed as well the leaf sheaths and meristematic regions of many of the older leaves. As a result of this injury the crowns, which in December were rather large in diameter, became smaller following each freezing. Finally but one or two leaves were left which were capable of elongation. The contrast was clearly seen in the hardier Minhardi, grown in the adjacent firm ground. Here the tendency toward radial depletion of the crown tissue was very markedly less, and in the spring more leaves could grow. The depletion of the storage in these two ways (by growth of new leaves and by killing of partially depleted tissues) resulted in weakened and non-hardy plants which rapidly decreased in hardiness in the greenhouse. Thus, on March 17 the remaining leaves of many of the Wisconsin Ped. no. 2 plants had been killed. In some cases a slight growth of perhaps half an inch was made, but most of these leaves finally failed to survive. In no case was growth from small axillary buds observed; that is, killing of the somewhat expanded leaf tissue at the crown appeared to kill the plant. Following March 17, decrease in hardiness was rapid, and almost complete in one week. Most of the Wisconsin Ped. no. 2 plants died, and the Minhardi plants showed rapid foliar elongation in the greenhouse.

It is interesting to note that on February 18 the plants of both varieties, as brought in from the field in an almost completely defoliated condition, were hardier than they were on December 18. They decreased in hardiness, however, much more rapidly in the week following February 18 than following December 18 (or January 18). This appeared to be associated with the two factors previously discussed. Growth of the leaves was not evident following December 18 or January 18, whereas it was rapid following

February 18. The plants brought in on February 18 had been almost completely defoliated on January 31, and had made about 1 inch of growth since that time; in the greenhouse these new leaves elongated quickly. Furthermore, the crowns had then been sufficiently injured so that some loss of leaf sheath tissue through killing had occurred.

Experiment 2

On January 22, lumps of soil containing plants of Minhardi (hardy) and Trumbull (rather tender) wheat were brought into the cold room from the field plats and stored at a temperature of 31°–36° F. The lumps of soil were sometimes partly frozen during the hours in the dark and sometimes completely thawed during illumination. One set of plants received light for 8 hours each day from a 100-watt lamp suspended about 15 inches above the plants. Another set was kept in darkness. After about a month the two sets were tested for hardiness, to see whether the plants would lose in hardiness more rapidly in darkness than in light. Table II shows that this is the case with both varieties. The samples stored in darkness at a temperature slightly above freezing showed marked elongation of etiolated leaves. This growth, combined with the lack of opportunity for photosynthesis, seems responsible for the rather definite decrease in hardiness observed. Trumbull plants decreased in hardiness more than Minhardi ones under this treatment. This might well be expected from the work of NEWTON and ANDERSON (7), who showed that the respiration rates of tender varieties of wheat are higher at low temperatures than are those of hardy varieties.

Part of the plants in both the light and the dark were defoliated on February 18 and kept in the cold room as before. In each case new leaves developed, although in some cases the lumps of soil were almost solidly frozen during the entire experiment. When these plants were tested for hardiness about a month later, plants which had been defoliated were more tender than undefoliated ones in each case.

On February 18, Minhardi plants in the field plats were defoliated by clipping the leaf blades with scissors. These plants promptly began foliar development. On March 17, following a period of cold weather, it was evident from the appearance of the plants that they had been severely injured by the cold. Every trace of the new green leaves was gone, and the plants were withered back to short stumps at the crown. The adjacent plants that had not been defoliated still had an abundance of green leaves. Samples were taken for the freezing-exosmosis test, and as the values in table II show, the defoliated plants were found to be much more tender than the corresponding undefoliated plants. The later development of these two sets showed that, while most individuals of both sets survived,

the defoliated plants were weak and slow to make new growth. While not winter-killed, they were certainly winter-injured.

TABLE II

SPECIFIC CONDUCTIVITIES ($\times 10^6$, 2° C.) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS OF CROWNS OF WINTER WHEAT FROZEN FOR 2 HOURS AT −15° C. INTERVAL OF 16 HOURS ALLOWED FOR EXOSMOSIS AT 2° C.

VARIETY	TREATMENT	READINGS TAKEN FEBRUARY 18
Minhardi	Outside, no serious injury by cold	266
	Inside, light, cold for one month	241
	Inside, dark, cold for one month	292
Trumbull	Outside, injured by cold on January 30	490
	Inside, light, cold for one month	430
	Inside, dark, cold for one month	567
		READINGS TAKEN MARCH 17
Minhardi	Outside, not defoliated with scissors	343
	Outside, defoliated with scissors on February 18	502

Discussion

The decrease in hardiness of winter wheat plants, so that they are more readily injured by cold weather, is a matter of considerable agronomic importance. The retention of hardiness seems, from these experiments, to be dependent upon the preservation of an adequate supply and concentration of organic foods. This supply is ordinarily depleted by respiration. If there is opportunity for the supply to be renewed through photosynthesis, as was the case in these experiments from December 18 to January 29, the plants may not become progressively more tender. Defoliation of the plants, whether artificially or by freezing injury, does not seem to be detrimental to their hardiness, as the conductivity values for February 18 show. Defoliation by either means, however, appears markedly to stimulate production or elongation of new leaves, which is provocative of rapid decrease in hardiness, presumably because of the labilization and use of the organic food reserves. The temperature at which this elongation takes place may be a decided factor in the effect produced. Thus the leaves of plants kept in darkness at about 0° C. elongated considerably in the course of a month, but without especially rapid decrease in hardiness of the plants. Plants elongating no more (and in fact in some cases imperceptibly, as from December 18 to 25) decreased materially in hardiness when kept at higher temperatures (about 17° C.) for one week. In general it would appear that the maintenance of the hardened condition in winter wheat plants is dependent upon environmental conditions which favor the con-

servation of organic food reserves; that is, which depress respiration and top growth and favor dormancy with continued periods of photosynthesis.

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FURTHER EVIDENCE THAT BORON IS ESSENTIAL FOR THE GROWTH OF LETTUCE[1]

J. S. McHargue and R. K. Calfee

(WITH EIGHT FIGURES)

In a previous report (**2**) it was shown that boron is essential for the growth of several varieties of lettuce; and that when it was excluded from the mineral nutrient solution, a severe deficiency disease resulted which was characterized by malformation of the more rapidly growing leaves, spotting and burning of the leaf tips, and death of the growing point of the plant. A similar condition of lettuce was described by Stone and Smith (**3**) as "top-burn." They considered the cause of the disease to be physiological and promoted by unfavorable surroundings. Le Clerg (**1**) measured leaf temperatures, but could not establish a relation between temperature and tip burning. The writers have further investigated the disorder, from the standpoint of a nutritional deficiency.

The influence of various boron compounds in preventing the burning of lettuce leaves was studied in both sand and water cultures. Control of the exact boron content of cultures was obtained by the addition of definite quantities of pure boron compounds to boron-free media. A dilute Pfeffer's solution, to which small quantities of manganese, copper, and zinc were added, supplied the basal mineral nutrients for both sand and water cultures. The salts composing the basal solution were proved to be free of boron by spectroscopical examination. Sand was purified by digestion with hot hydrochloric acid, followed by removal of chlorides with distilled water. Distilled water required for the preparation of cultures, growth of plants, purification of sand, and rinsing of containers was condensed in quartz. Porcelain dye pots and special acid-resistant jars with perforated lids were used as containers for the sand and water cultures respectively. No evidence of boron contamination from the use of these containers was observed.

Lettuce seeds were germinated in purified sand, and transferred to sand or water cultures containing, with the exception of boron, all elements known to be essential for plant growth. Severe boron deficiency, as indicated by severe injury to the leaf tips, developed in from two to four weeks, depending upon the variety, amount of light, and type of culture used.

[1] Contribution from the Department of Chemistry of the Kentucky Agricultural Experiment Station.

The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

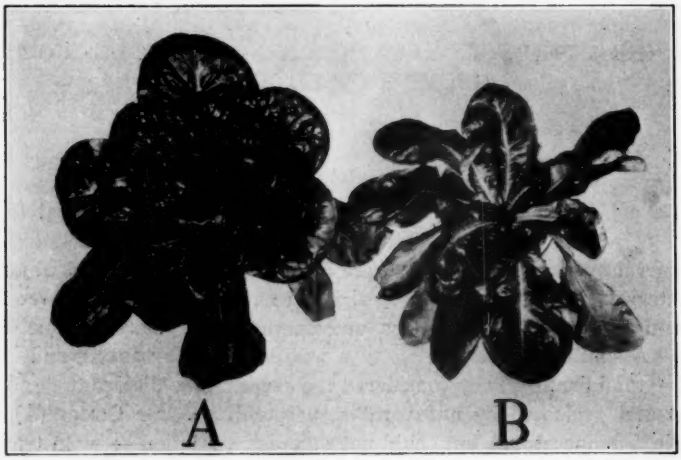

FIG. 1. Effect of boron on growth and development of lettuce plants: *A*, plant grown in sand containing 0.5 p.p.m. boron; *B*, plant not supplied with boron.

The first symptoms of boron deficiency were a retardation of growth and malformation of the younger leaves. Dark spots then appeared on the margin of the growing leaves, usually at the tip. Marginal growth was

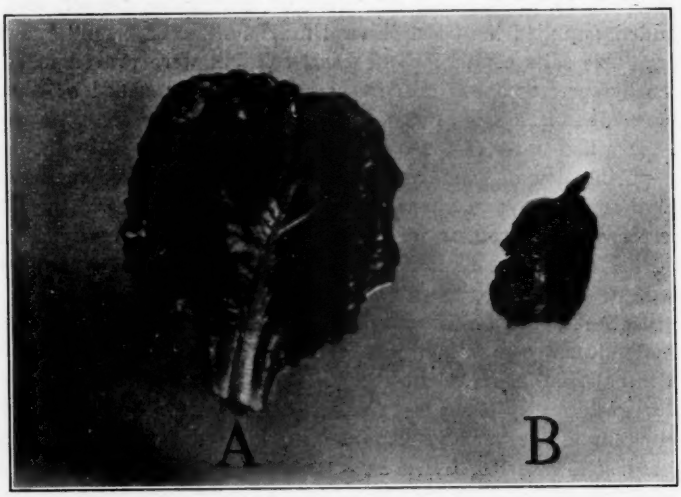

FIG. 2. Effect of boron deficiency on leaf development: *A*, leaf from normal plant; *B*, leaf from plant showing severe boron deficiency.

suspended, resulting in a folding back of the leaf tip. Normal and deficient plants at this stage are shown in figure 1. The spots increased in size and number, involving the entire leaf tip and giving it a scorched appearance. Figure 2 shows leaves from normal and deficient plants of the same age. The older leaves are not noticeably affected by the absence of boron; but all young leaves, from those first affected to the growing point itself, are involved, resulting finally in the destruction of the meristem tissue and consequently in the death of the plant (fig. 3).

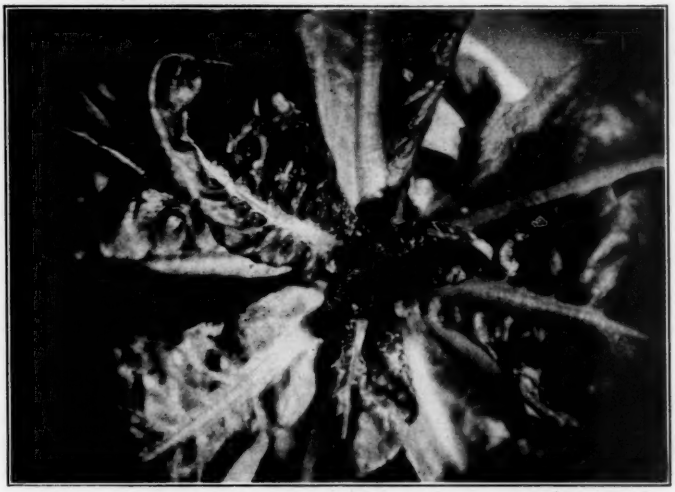

FIG. 3. Growing point of lettuce plant showing severe boron deficiency.

The addition of a small quantity of boron to the culture before the death of the growing point relieved the condition and resulted in the production of normal leaves from the growing point. The addition of boron compounds after the death of the growing point resulted in growth from lateral buds in the leaf axils. Figures 4–6 show the results of continued additions of boron to the media after the development of severe deficiency, allowing the plant to make very satisfactory growth to maturity. Plants that were retarded in early growth by the absence of boron did not attain the same size or weight at maturity as did normal plants (positive controls).

Small quantities of boric acid, boro-silicate (powdered Pyrex glass), and borates of potassium, sodium, calcium, manganese, copper, and zinc were found to be effective in preventing injury to lettuce leaves.

To ascertain the optimum concentration of boron for the growth of lettuce, cultures in triplicate were treated with boric acid varying the boron

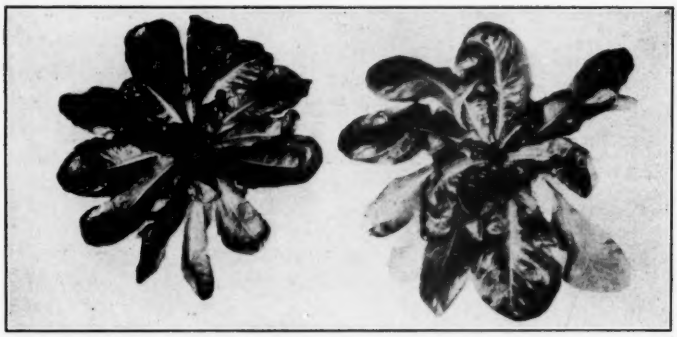

FIG. 4. Lettuce plants in which boron deficiency had caused death of the growing point. Boric acid was added to the one on the left at this time.

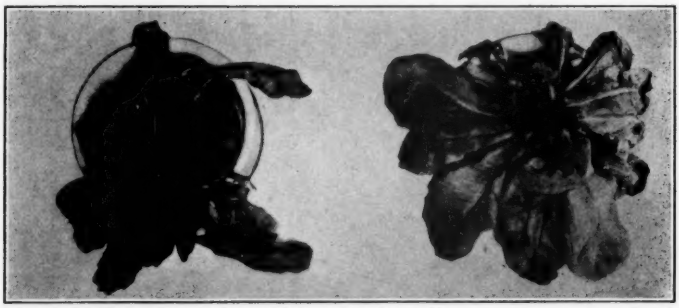

FIG. 5. Same plants as in figure 4, 14 days later. Growth has been resumed from axillary buds in the plant receiving boric acid.

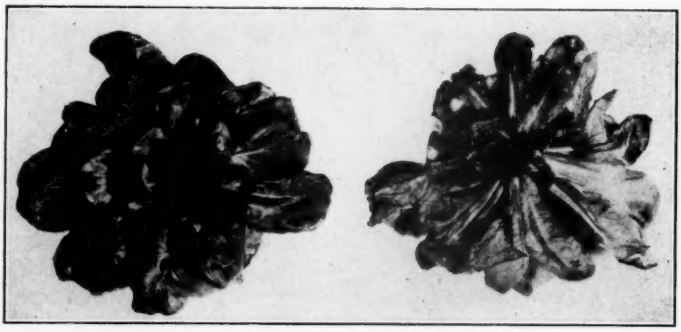

FIG. 6. Same plants as in figure 4, 6 weeks after addition of boric acid to the one on the left.

content by 0.1 p.p.m. from no boron to 1.5 p.p.m. The range between 1.5 and 3.0 p.p.m. was covered by increase of 0.3 p.p.m. boron.

Plants that did not receive boron showed injury shortly after being transferred to the experimental cultures. With 0.1 and 0.2 p.p.m. boron in the medium, plants made much slower growth than those receiving larger quantities, within non-toxic range. The presence of 0.3 p.p.m. boron in the nutrient solution enabled plants to make continued growth until shortly before reaching maturity; then during periods of rapid growth injury would appear on the more rapidly growing leaves. During periods of lower activity, imposed by shading or unfavorable weather conditions, growth would be resumed, with the production of apparently normal leaves. The plants of this group were much smaller than normal plants and did not produce flowers. All plants in the group grown in a boron concentration of 0.4 p.p.m. were free from leaf injury. Florescence occurred although

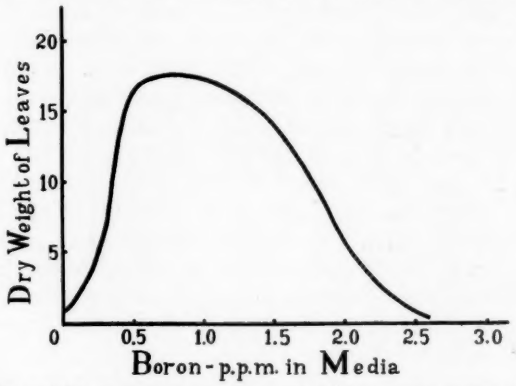

FIG. 7. Dry weight, in grams, of leaves of lettuce plants grown in the presence of different quantities of boric acid.

but few seeds were produced. Quantities of boron between 0.4 and 0.9 p.p.m. in the culture resulted in the production of vigorous, rapidly growing plants with no evidence of toxicity or boron deficiency. Seed pods containing normal seeds were especially numerous on plants grown in concentrations of 0.6 and 0.7 p.p.m. boron. Nine-tenths of a part of boron per million was slightly toxic to lettuce, resulting in perceptible chlorosis of the lower leaves in most plants. The chlorosis was more pronounced with 1.0 p.p.m. boron in the nutrient solution. Boron concentration of 1.2 p.p.m. was decidedly toxic, causing chlorosis and death of the older leaves and large, white, necrotic spots on the edges of mature living leaves. All concentrations of boron from 1.2 to 2.5 p.p.m. produced increasing toxicity, as shown by necrosis and a significant decrease in the size of the plants.

Quantities of boron exceeding 2.5 p.p.m. were fatal to the seedlings. The effects of different quantities of boron on the growth of lettuce as determined by dry weight of the leaves are shown in figure 7. On the basis of dry weight, the optimum concentration of boron in water cultures for the growth of lettuce is 0.7 p.p.m. added in the form of boric acid.

The utilization of various boron compounds by lettuce was investigated in sand cultures to determine the relative amounts that must be present for normal growth. The powdered borates were mixed with purified sand in porcelain dishes before placing in the containers. Other mineral nutrients were added in solution as required through the period of growth. Treatments and results are given in table I.

Additions of boric acid, or the relatively soluble borates of potassium, sodium, and calcium to sand in sufficiently small quantities to avoid toxic effects were insufficient for the later growth requirements of the plants, unless a large volume of medium was used. Normal plants could not be grown in sand cultures of 750 or 1500 gm. without further additions of soluble boron compounds during growth. Cultures of 5 kg. containing sufficient soluble boron compounds to produce normal plants were not toxic to plants started during the early summer, but were decidedly toxic to seedlings started during periods of more limited light intensity and shorter daily exposure. The quantity of soluble borate in cultures of 10 kg. was decreased to a content that was non-toxic to seedlings at any period of the year without the appearance of deficiency symptoms in later growth.

The inclusion of 0.0025 gm. of boron as manganese borate in sand cultures was sufficient for the growth to maturity of the lettuce plant. Relatively large quantities of manganese borate were not toxic to plants in cultures having reaction exceeding pH 6.6. Increase in the acidity of cultures resulted in high concentrations of manganese borate becoming toxic. Toxicity resulting from an excess of manganese borate was due to the boron ion. The addition to sand cultures of sufficient copper borate to supply the plants' boron requirements throughout the period of growth resulted in toxic concentrations of the copper ion.

Boro-silicate in the form of powdered Pyrex glass was found to be the most satisfactory source of boron for sand cultures. Quantities sufficient for the successive growth to maturity of several plants were included in the medium without toxicity or the development of deficiency symptoms. Slight changes in the reaction of the culture did not affect the solubility of the boro-silicate.

The ratio between the quantity of boron available and the quantity absorbed and its relation to the physical condition of the plants was ascertained by determining the boron content of plants grown in water cultures

TABLE I

EFFECTS OF DIFFERENT QUANTITIES OF BORON COMPOUNDS ON GROWTH OF LETTUCE

COMPOUND	BORON PER KG. SAND	EFFECT OF BORON COMPOUND ON PLANT
	gm.	
Boric acid	0.0005	Deficiency severe
	0.0010	Deficiency severe
	0.0015	Slightly toxic, final deficiency
	0.0020	Toxic
Potassium borate	0.0005	Deficiency severe
	0.0010	Deficiency severe
	0.0015	Deficiency; some toxicity
	0.0020	Toxic
Sodium borate	0.0005	Deficiency severe
	0.0010	Deficiency severe
	0.0015	Deficiency
	0.0020	Toxic
Calcium borate	0.0010	Deficiency severe
	0.0015	Deficiency
	0.0020	Slightly toxic at first; good growth
	0.0025	Toxic
Manganese borate	0.0025	Good growth
	0.0075	Very good growth; plant normal
	0.0100	Slightly toxic
	0.0150	Boron and manganese toxic
Copper borate	0.0010	Deficiency severe
	0.0025	Cu. slightly toxic
	0.0050	Cu. toxic; severe
	0.0075	Seedlings killed
Zinc borate	0.0010	Deficiency severe
	0.0050	No deficiency or toxicity
	0.0100	Zn. toxic, slight
	0.0150	Zn. toxic, severe
Boro-silicate (powdered Pyrex glass, 40 mesh)	10.0	Plant normal
	25.0	Plant normal
	50.00	Very slightly toxic
	100.00	Definitely toxic

at constant concentrations of boron. The boron content of normal lettuce varied between 25 and 50 p.p.m. boron of the moisture-free tissues. All plants containing less than 20 p.p.m. boron showed some degree of defi-

ciency. Boron toxicity occurred in all plants containing more than 60 p.p.m. boron.

Figure 8 shows the effects of different concentrations of boric acid in

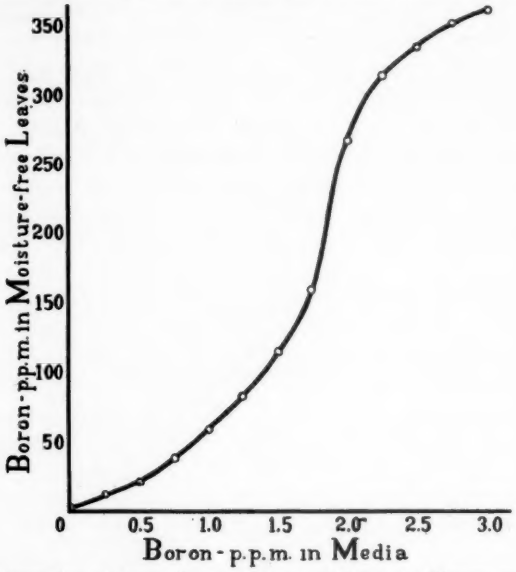

FIG. 8. Absorption of boron from nutrient solutions of different concentrations of boric acid.

the nutrient solution on the absorption of boron by lettuce. The concentration in the leaves depended upon the concentration of soluble boron in the solution. Slight increases in the boron content of a solution exceeding toxic concentrations resulted in large increases in the boron content of the leaves. Additions of boric acid greater than the quantity sufficient to kill the seedlings rapidly caused a slightly greater increase in the boron content of the leaves than did the minimum lethal concentration, indicating a relation between the concentration of boron in the medium and the rate of absorption by the plant.

Summary

1. A deficiency disease of lettuce resulting from an insufficiency of boron is described and illustrated.

2. The effectiveness of several boron compounds in preventing or correcting the deficiency disease is given.

3. The concentrations of soluble boron compounds that result in boron deficiency, normal growth, and toxicity were ascertained.

4. The physical condition of the plant was modified by the concentration of soluble boron in the nutritive solution.

5. The effect of the less soluble boron compounds was modified by the reaction of the culture, the volume, and, through the plant itself, by climatic and seasonal conditions affecting the rate of growth.

6. Boro-silicate was found to be the most satisfactory compound for incorporation in sand cultures.

7. Increase in the boron content of the nutrient solutions up to concentrations that rapidly resulted in the death of the seedlings produced increasingly greater concentrations of boron in the leaf tissue.

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RAPID COLORIMETRIC DETERMINATION OF POTASSIUM IN PLANT TISSUES[1]

V. H. Morris and R. W. Gerdel

Introduction

One of the most important factors limiting studies of the mineral nutrition of plants is the time and labor consumed in the analytical processes. Nowhere in the field of plant physiology is the need for rapid, convenient, and accurate methods more keenly felt. With such material as corn, the great variability in the composition of individual plants of even nominally pure lines is such as to necessitate considerable replication of samples in order to obtain analyses which are representative of the population.

Of the plant constituents usually considered in mineral studies, potassium is probably the most difficult and tedious to determine by the conventional methods. Both the chloroplatinate and perchlorate methods are time-consuming and involved. In recent years, however, a method based on the precipitation of the potassium by sodium cobaltinitrite has met with increasing favor, particularly in blood and urine analyses. A study of the adaptability of this method to samples of plant tissues, of which corn may be considered representative, is reported in this paper.

Method

The principle of the method is based upon the precipitation of potassium as the double salt, potassium sodium cobaltinitrite, of the general composition $K_2NaCo(NO_2)_6 \cdot H_2O$. The quantity of the precipitate may be determined by any of the three common analytical procedures, gravimetric, volumetric, or colorimetric. Since colorimetric methods are notably time- and labor-saving, while retaining a reasonable degree of accuracy, such a procedure seemed most desirable.

As with most methods for determining potassium, the presence of ammonia in the sample interferes by forming a relatively insoluble precipitate with the reagent. Ammonia must accordingly be removed from samples containing enough to introduce serious error. Seasonal studies have shown that the quantity of ammonia present in corn tissue at any time is not sufficient to introduce appreciable error. This is probably true also of most plants, except perhaps when they are grown under unusual environmental conditions.

[1] Investigations cooperative between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture and the Department of Agronomy, Ohio Agricultural Experiment Station.

For use with corn the method must be adaptable to two kinds of samples, sap expressed from fresh, succulent tissues, and solutions of the ash from dry, mature tissues. As determined by the chloroplatinate method, sap expressed from different tissues of the corn plant contained from 1 to 6 mg. K per ml. Ashed solutions contained comparable quantities in 25 ml. of the solution as prepared for analysis.

Three procedures for colorimetrically measuring the quantity of the precipitated potassium were investigated: (a) The method suggested by EMMERT (**1**), based upon measuring the change in strength of the precipitating reagent. With us the method appeared incapable of giving the desired degree of accuracy. (b) Methods based upon determining the quantity of nitrite present in the precipitate. Several of the methods described by YOE (**4**) were tried. The principal objection to these methods is that they are very sensitive to small quantities of nitrites, thus making it necessary to use very dilute solutions in which to develop the color. These methods are open also to the theoretical objection that the nitrite radical may be considered relatively unstable. (c) Methods based upon estimating the quantity of cobalt in the precipitate. The method described by YOE (**4**), involving the use of α-nitroso β-naphthol, was open to the same objection as that to the nitrite methods,—the necessity of developing the color in too dilute solutions. The method suggested by JACOBS and HOFFMAN (**2**) was then tried and found to be well adapted to the material in hand. This method is based upon the development of a brilliant emerald green color by the addition of ferrocyanide to a solution containing choline and a cobaltous salt.

Procedure

The precipitation and determination of potassium were carried out as follows:

The expressed sap, obtained as described in a previous paper (**3**), was cleared by warming to 55° in a water bath, shaking with about 1 gm. of carbon black per 100 ml., and filtering. To 1 ml. of the clear filtrate in a 50-ml. lipped centrifuge tube were added 2 ml. of EMMERT'S (**1**) sodium cobaltinitrite reagent.[2] The tube was allowed to stand for 15 to 20 minutes, with occasional shaking. About 10 cc. of 70 per cent. alcohol were added from a wash bottle and the tube centrifuged for several minutes at moderate speed. The supernatant liquid was poured off, allowing time for

[2] EMMERT'S sodium cobaltinitrite reagent: Dissolve 5.1666 gm. of pure sodium cobaltinitrite in 40 ml. of distilled water; dissolve 27 gm. of potassium-free sodium nitrite in another 40 ml. of distilled water; pour the solutions together, add 6 ml. of glacial acetic acid, and make up to 100 ml. with distilled water. One ml. will precipitate about 10 mg. of K as $K_2NaCo(NO_2)_6 \cdot H_2O$. The solution evolves gas when first prepared but this does not impair its value.

thorough drainage. This washing process was repeated twice more, using about 10 cc. of 70 per cent. alcohol each time. After the final centrifuging, the precipitate was washed into a 100-ml. volumetric flask with water and dissolved by heating on a steam plate. After cooling to room temperature the solution was made up to volume and shaken. Twenty-five ml. of the solution were pipetted into an Erlenmeyer flask, 5 cc. of a 1 per cent. choline hydrochloride solution and 5 cc. of a 2 per cent. potassium ferrocyanide solution being added with shaking. A brilliant emerald green color develops instantly, the intensity of which is directly proportional to the quantity of cobalt present. The color was compared in a Klett colorimeter with a standard prepared by precipitating a solution of KCl containing 2 mg. K per ml. and carrying it through the same procedure as with the unknown. This method of standardization is necessary, since it has been shown by previous workers that the composition of the precipitate with respect to the sodium content is influenced somewhat by the relative quantities of potassium and precipitating reagents.

With the dried tissues, 2.5-gm. samples were ashed with sulphuric acid, dissolved in dilute HCl, filtered, and diluted to 250 ml. Twenty-five ml. of this solution were evaporated to dryness in a 100-ml. beaker, 1 ml. of water added, and, after the salts were again in solution, 2 ml. of the reagent added. After transferring the precipitate to a 50-ml. centrifuge tube with 70 per cent. alcohol, the procedure was the same as with the sap samples.

Results

The method has been tried on several materials with results as shown in table I. The recovery of potassium from solutions containing different quantities of potassium acid phthalate and potassium chloride was satisfactory, the error in no case exceeding 0.5 mg. The results of a comparison of the colorimetric method with the standard chloroplatinate method as applied to a series of expressed sap samples are also recorded in the table. The samples were obtained during a study of the comparative potassium contents of a group of corn hybrids, each sample representing the stem tissue of a different hybrid. The samples were taken in duplicate and the values given in the table are the average of single determinations on the duplicates by the method stated. The means for the two methods are in very good agreement. Although the difference between duplicates was rather large in some cases, there is no reason to assume differential variation between samples determined colorimetrically and those determined gravimetrically. Consequently, the difference in the variances for the two methods may be interpreted as indicating that the colorimetric method was at least as precise in these experiments as the chloroplatinate method. Likewise, when the two methods were applied to expressed sap samples from

TABLE I

RECOVERY OF POTASSIUM FROM KNOWN SOLUTIONS BY COLORIMETRIC METHOD AND COMPARISON OF COLORIMETRIC AND GRAVIMETRIC CHLOROPLATINATE METHODS WITH DIFFERENT KINDS OF SAMPLES

RECOVERY OF K FROM KNOWN SOLUTIONS		SAMPLE, HYBRID	EXPRESSED SAP SAMPLES OF CORN STEM TISSUES		SAMPLE	EXPRESSED SAP SAMPLES OF DIFFERENT TYPES OF CORN TISSUE		SAMPLE, HYBRID	SOLUTIONS OF ASHED CORN STEM TISSUES	
KCl TAKEN	KCl FOUND		COLORIMETRIC	GRAVIMETRIC		COLORIMETRIC	GRAVIMETRIC		COLORIMETRIC	GRAVIMETRIC
mg. K	*mg. K*		*mg. K per ml. sap*	*mg. K per ml. sap*		*mg. K per ml. sap*	*mg. K per ml. sap*		*mg. K per 0.25 gm. tissue*	*mg. K per 0.25 gm. tissue*
5.2	5.0	1	3.6	3.0	Blade	4.2	4.2	25	2.4	2.4
2.6	3.1	2	3.0	2.9	Sheath	2.8	2.4	26	3.7	3.4
1.1	1.1	3	2.5	2.9	Upper stem	2.0	1.7	27	3.1	3.0
2.1	2.2	4	1.8	1.9				28	3.0	2.4
3.1	3.1	5	2.9	2.9	Lower stem	1.5	1.4	29	2.1	2.0
4.2	4.4	6	2.3	2.6				30	2.4	2.7
5.2	5.2	7	2.3	2.4	Tassel	3.2	3.0	31	3.5	3.5
		8	2.0	2.0				32	2.6	3.0
		9	2.1	2.1				33	4.2	4.5
		10	3.2	3.1				34	3.5	3.5
		11	4.1	3.8				Mean	3.1	3.0
		12	2.5	3.2						
		13	2.9	3.9						
		14	3.0	2.6						
		15	2.5	3.1						
$KHC_2H_4O_4$ TAKEN	$KHC_2H_4O_4$ FOUND	16	4.3	4.5						
		17	3.5	3.2						
		18	1.6	2.0						
mg. K	*mg. K*	19	2.2	1.8						
5.7	5.3	20	2.4	2.5						
2.9	3.1	21	4.3	4.4						
5.7	5.6	22	3.4	3.1						
		23	3.0	2.8						
		24	2.0	1.6						
		Mean	2.8	2.9						
		Variance	0.128	0.184						

different tissues of the corn plant taken on the same date, satisfactory agreement between the two methods was obtained.

A comparison of the two methods as applied to ashed samples indicates that the colorimetric method also may be used with this type of sample.

The colorimetric method is not only much cheaper from the standpoint of cost of chemicals and equipment, but is also advantageous from the point of time consumed. At least 24 determinations may be completed in 8 hours, starting either with expressed sap or with ashed solutions, whereas the chloroplatinate method would require 16 to 32 hours for the same number. When working with variable plant material, therefore, the opportunity is afforded for using a greater number of replicates to obtain much greater final precision.

Summary

The colorimetric procedure of JACOBS and HOFFMAN for determining potassium precipitated as the cobaltinitrite affords a rapid and convenient method for samples of plant tissue. The method compares very favorably with the chloroplatinate method with respect to precision; and the saving of time and labor, permitting the handling of a larger number of replicates, results in a much greater final precision.

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A NEW METHOD AND INSTRUMENT FOR THE QUANTITATIVE DETERMINATION OF CHLOROPHYLL[1]

R. E. OLTMAN

(WITH THREE FIGURES)

In research problems in plant physiology, it occasionally becomes desirable to make a considerable number of quantitative determinations of chlorophyll. The method by which this is done should be one that is speedy, open to a minimum of error, and not so unwieldy as to be difficult for the general botanist to employ. Many methods for determining the amount of chlorophyll present in leaves or in solutions have been suggested, but only two have been found to be of sufficient accuracy to permit a quantitative comparison with the results of other workers. SCHERTZ (**2**) has described the colorimetric and the spectrophotometric methods, and has shown the percentage of error prevalent in each.

The colorimetric method depends on the ability of the eye to match depths of color, an operation in which the human eye is notoriously inefficient. In addition, this method does not adequately determine slight differences in color, the limit of sensitivity and accuracy being two decimal places.

The spectrophotometric method depends on measuring the width of the absorption bands of chlorophyll in the red end of the spectrum. This method is neither speedy nor practical for making a considerable number of determinations, since for each determination a separate spectrophotograph must be taken, which requires considerable time. In addition, an expensive spectrograph is necessary, and the aid of an expert physicist is advisable for correctly interpreting the photographs. Like the colorimetric method, it is possible to ascertain concentrations of chlorophyll only to two decimal places.

The purpose in developing the instrument here described was to eliminate the possibilities of personal error in observation, to detect more minute differences than is possible by the preceding methods, and to make the quantitative determination of chlorophyll an easier and simpler process. This instrument will detect differences of 5 mg. between samples, and has a maximum error of 9 per cent. at the lower concentrations and a minimum error of 2 per cent. at the higher concentrations, which errors in most cases affect only the third decimal place.

Figures 1 and 2 illustrate the apparatus. A photoelectric cell (Weston photronic), which requires no external resistances, no amplification of the

[1] Presented at the general scientific sessions of the Ohio Academy of Science, Delaware meeting. April, 1932.

Papers from the Department of Botany, Oberlin College, no. 4.

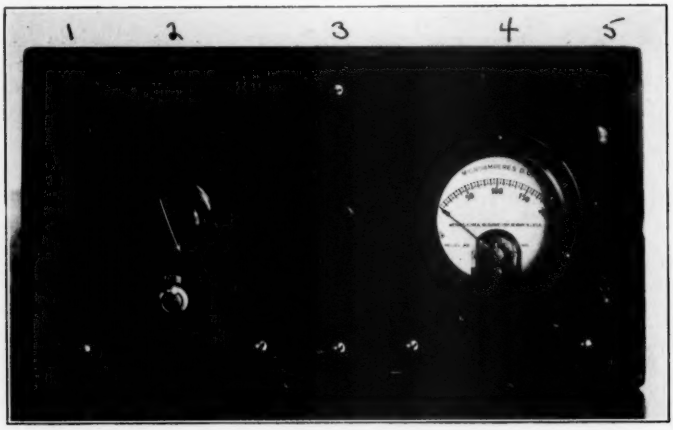

FIG. 1. Exterior view of instrument: *1*, binding posts for attachment of 6-volt storage battery; *2*, rheostat and switch; *3*, sliding panel; *4*, microammeter; *5*, output binding posts from microammeter.

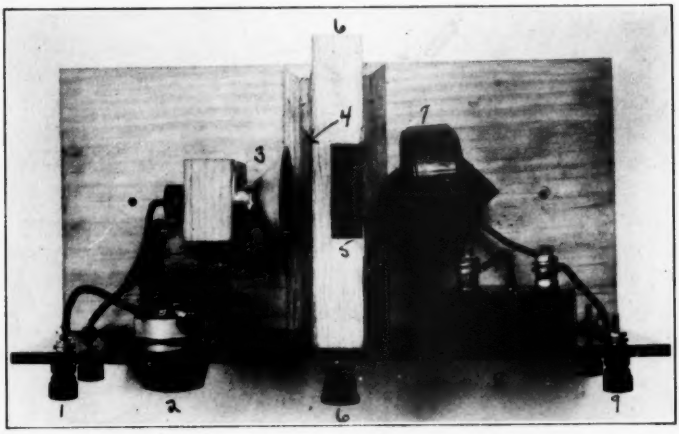

FIG. 2. Interior view of instrument: *1*, binding posts for 6-volt battery connection; *2*, 4-ohm rheostat; *3*, 2-candle-power automobile bulb; *4*, light filter (Corning Lantern shade yellow, no. 349); *5*, glass absorption bottle fitted in groove in panel; *6*, sliding panel (note guides on sides); *7*, photoelectric cell (Weston photronic, Model 594) mounted in UX type radio socket; *8*, microammeter (Weston, Model 301); *9*, output binding posts from microammeter, so that meter can be used for other purposes without dismounting.

current emitted, and which emits a current in direct proportion to the light energy absorbed on its face (over rather wide limits) is employed. This cell is mounted upright in a UX type radio socket, 15 cm. from a 2-candle-power, 6-volt automobile bulb fitted with a small reflector. A rheostat to vary the light intensity is connected in series with a well-charged 6-volt storage battery and the small automobile lamp. The position of the lamp is so adjusted as to place its center directly opposite the center of the face of the photoelectric cell. The current emitted by the photoelectric cell when exposed to light is measured by a microammeter with a range of 0–200 microamperes, which is connected to the leads of the UX type socket into which the cell is fitted. Between the cell and the lamp a sliding panel of thick wood is interposed, with a 1.5-inch hole bored through it on a direct line with the center of the lamp and the photoelectric cell, so that the light from the lamp is allowed to pass through and strike upon the face of the cell.

On one side of this sliding panel a 2-inch square light filter, transmitting 5600–7000 Å. (Corning Lantern shade yellow, no. 349), is mounted over the hole in the panel. By this means the light from the lamp is all converted to those wave lengths which WURMSER (**5**) and others have shown to be most strongly absorbed by chlorophyll. The red light is then passed through a small glass absorption cell fitted flush to the panel in a groove on its other side. This absorption cell has an inside thickness of 1 cm., so that the red light passes through a 1-cm. layer of liquid when the cell is filled.

To take a reading, the absorption cell is first filled with distilled water and the light switched on. The intensity of the light on the face of the photoelectric cell is rapidly adjusted by means of the rheostat so that the needle of the microammeter swings to exactly 200 microamperes, the maximum of the meter. Speed is essential in doing this, since even a small lamp is sufficient to "run down" the battery enough to cause an appreciable change in the light intensity of the bulb. Although this change is not visible to the eye, it is apparent upon the dial of the meter. When the light intensity has been satisfactorily adjusted, so that the needle of the meter will swing back to the maximum each time the light is switched on, the light is turned off and the absorption bottle filled with the water solution of potassium chlorophyllin to be measured. The light is switched on, and the decrease in the light intensity transmitted, due to the absorption of light by chlorophyll and measured by the microammeter, is observed. If purely comparative results are desired, the readings obtained will give an accurate means of comparison,—the smaller figures representing the greater absorption and hence the greater concentration of chlorophyll.

The method of preparation of the chlorophyll solutions previous to their quantitative determination is essentially that of WILLSTÄTTER and STOLL

(4), with more recent modifications suggested by SCHERTZ (3) and MORROW (1). Since several slight alterations in procedure have been made, the method is briefly outlined as follows:

The leaves are cut as free of petioles as possible, and their fresh green weight determined. They are then dried in an electric oven at 37° C., pulverized in a mortar, and the powder further dried to expel any remaining moisture. The time required for complete drying varies with the thickness of the leaves,—in most cases three or four days will suffice. The dry weight is then taken and the percentage of solid matter determined. One-half gm. of the dry leaf powder, which amount requires less solvent and is more completely extracted in a shorter time than a larger amount, is placed in a Soxhlet siphon extractor, and continuously extracted for 24 hours with a 1:1 mixture of ether and acetone. The solvent flask is immersed in water

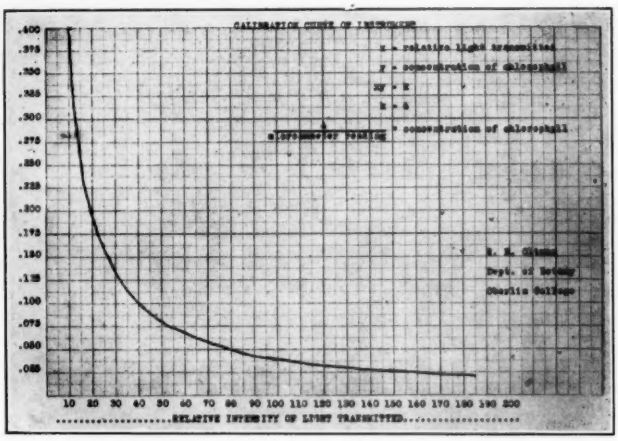

FIG. 3. Calibration curve of instrument.

kept at a temperature not exceeding 50° C. by means of an electric hot-plate. The solution is then placed in a separatory funnel, the acetone washed out with water, the anthocyanins and flavones with 1 per cent. sodium carbonate solution, and the ether solution washed twice more with water. The ether solution containing chlorophyll (alpha and beta) carotene and xanthophyll is then treated with 10 cc. of methyl alcohol saturated with KOH, and placed on ice for 24 hours. The potassium chlorophyllin separates out from the ether solution, leaving a clear golden-yellow liquid which contains the carotene and xanthophyll. By washing with water, the two layers are separated; the potassium chlorophyllin solution is washed *once* with ether and made up to 100 cc., at which point in the procedure it is ready for measurement in the instrument.

To calibrate the instrument so that the meter readings will be an actual indication of the amount of chlorophyll present in a solution, a series of solutions of known concentrations are made up from chemically pure chlorophyll. The chlorophyll is dried in a vacuum desiccator over sulphuric acid for a week, and ten accurate weighings made. When dry the chlorophyll is dissolved in ether, converted into its potassium salt by saponification with methyl KOH, and 40 different concentrations made up by dilution. The actual curve is constructed from the 40 readings obtained with these solutions in the instrument, with the x axis representing the microammeter readings (relative light transmitted) and the y axis the concentrations of chlorophyll in grams per 100 cc. This curve is found to be an hyperbola, with the equation $xy = k$.

The constant k for the 40 readings is averaged, and from the data already obtained in constructing the actual curve, an ideal curve is drawn. The calibration curve of the writer's instrument (fig. 3) differed only slightly from the actual curve first obtained. In this instrument, the constant k is found to be 4, so that

$ky = 4$ is the equation of the hyperbola, or

$$\frac{4}{\text{microammeter reading}} = \text{concentration of chlorophyll in grams per 100 cc.}$$

It must be emphasized that it will be necessary for each investigator to calibrate his own instrument and find his own ideal curve and equation, since no two photoelectric cells are exactly alike in their sensitivity to different wave lengths of light, or to different intensities of the same wave length. The purity of the chlorophyll samples used in calibration will also affect the constant k. In any case, however, the calibration curve will be an hyperbola, which approaches the x and y axes asymptotically, but the eccentricity depends on the constant k, and will vary according to the cell and the purity of the chlorophyll used in calibration.

Since 0.5 gm. of dry leaf powder has been used for extraction, the amount determined in the instrument is multiplied by two, which gives the amount of chlorophyll in 1 gm. of leaf powder. To refer this figure back to the green leaf material, it is multiplied by the percentage of solids in the green leaf, which has been determined when the leaves were dried. By dilution of the sample being determined, averaging the results after making allowance for the dilution will give a much more accurate figure than a single determination on each sample.

The instrument is not limited in its use to the quantitative determination of chlorophyll. It may be employed to match colors of dyes and to replace colorimetric methods now in use in many quantitative determinations. It can also be used to make certain *qualitative* color reactions *quantitative,* by employing light filters whose range corresponds to the strongest

absorption bands of the reaction product, and calibrating with solutions of known concentrations.

The writer wishes to acknowledge the helpful criticisms and suggestions of Dr. F. G. TUCKER and Mr. JAMES SNODGRASS of the Department of Physics of Oberlin College, and to express his appreciation to Professor F. O. GROVER for material aid in developing the apparatus.

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GASOMETRIC METHOD OF ESTIMATING OXIDASE ACTIVITY

R. N. JEFFREY AND W. V. CRUESS

In the study of biological oxidations, two criteria are used as indications of oxidase activity. One of these is the oxidation-reduction potential of the system concerned, determined as described by CLARK (**3**) and others. The other is the rate of oxidation measured by one of several methods. In our particular studies on the oxidase systems of various fruits, measurement of rate is much more useful than measurement of oxidation-reduction potential.

Rate measurements

Four important methods of measuring the rate of enzymic oxidation have been reported in the literature. These are the (1) colorimetric, (2) titrimetric, (3) electrometric, and (4) manometric methods.

If fruit oxidase is at all specific, it is very probable that the rate at which it oxidizes an added indicator is different from that at which it oxidizes the naturally occurring substrate. It is also possible that different catalysts are involved in oxidation of the natural and the added substrates. There are other objectionable features also, such as interference of turbidity of the juice, and difficulty in duplicating tints in permanent color standards.

Several of the more important colorimetric methods are those of DYE (**6**), RÖHMAN and SPITZER (**13**), and WILLSTÄTTER and WEBER (**17**). Most colorimetric methods require the addition of H_2O_2. This addition alters natural conditions, and CRUESS and FONG (**4**) have shown that its concentration greatly affects the results obtained.

GUTHRIE (**8**) uses in a titrimetric technique a special substrate formed by the action of NaOH on glucose solution. HAEHN and STERN (**9**) have reported upon a method in which unchanged added tyrosin is determined at intervals.

In the electrometric method of STEARN and DAY (**15**), hydroquinone of the quinone-hydroquinone complex used with the quinhydrone electrode is oxidized by the oxidase, changing the ratio of quinone to hydroquinone and thus the E.M.F. of the quinhydrone electrode. The resulting drift in potential is taken as a measure of the rate of oxidation.

One of the earliest applications of the manometric method was made by FOÀ (**7**). The well known BARCROFT manometric apparatus and its modified form, the WARBURG (**16**) apparatus, are in very general use. DIXON and ELLIOTT (**5**) have recently still further modified the BARCROFT apparatus. The BUNZEL (**1, 2**) apparatus also makes use of the manometric principle (see also SENNHAUSER **14**).

Practical objections to the manometric apparatus are its high cost and

the rather large high differential pressures developed. The volume of liquid used is also small, usually less than 5 cc.

Volumetric apparatus used

In the apparatus devised by the writers, a number of the objections to the colorimetric, titrimetric, potentiometric, and manometric methods are obviated. It is volumetric instead of manometric; hence the internal pressure need not exceed 1 mm. of Hg compared with pressures occasionally exceeding 50 mm. of Hg in the manometric method. There is, therefore, little tendency for leakage of gas outward or inward. The volume of the reaction flask may be much larger than in the manometric method, and it is not necessary that its volume be known accurately, since change in volume at atmospheric pressure is measured. Ground glass connections are not necessary, and since relatively large volumes of O_2 are absorbed during the course of an experiment, standard gas burettes may be used for measurement of the volume of gas absorbed. Also, since the volume of the flask and burette is much larger in proportion to the volume of substrate than is the case in the usual manometric apparatus, there is less danger of retardation of reaction rate because of O_2 absorption and reduction of the partial pressure of O_2. In building the apparatus common and relatively inexpensive laboratory equipment may be used.

The constant temperature bath is $18 \times 18 \times 8$ inches, and of galvanized sheet metal thermally insulated by asbestos. The water is brought to operating temperature by means of a 500-watt knife-type Cenco "lagless" heater, and is maintained at operating temperature by means of a similar 125-watt heater controlled by a mercury-filled electric regulator and small relay. The relay is operated by a 6-volt battery charged by a radio battery charger. It is possible to maintain the temperature within a range of 0.02° C.

Four 250-cc. Erlenmeyer flasks are held in position in the bath by clamps to two movable $\frac{3}{8}$-inch rods suspended above the tank. The rods are moved back and forth horizontally at the rate of 90 times a minute by means of a $\frac{1}{4}$-H. P. motor, geared down to the proper speed by wooden pulleys.

The four Erlenmeyer reaction flasks are fitted with rubber stoppers and connected to gas burettes outside the reaction chamber by means of heavy-walled, capillary glass tubing. The volume of the connecting capillary of each flask is less than 1 cc. A stop-cock connected to a small thistle tube fitted through the stopper of each flask is used to introduce added solutions and to equalize the pressure at the beginning of the run.

There is suspended inside each flask from the stopper a short wire holding a small glass cup in which is placed a small roll of filter paper, cut in

the form of a rosette at the top, and saturated with N/1 NaOH solution to absorb CO_2 liberated during the experiment. With fruit juices, the volume of CO_2 liberated is relatively large and must be removed in this manner in order to avoid serious error.

The four gas burettes are attached by clamps to the desk in front of the constant temperature bath. The top of each burette is slightly above the surface of the bath, in order that the connection to the reaction flask shall be as short as possible. The connecting capillary glass tubing is joined to the burette and to the flask by flexible rubber connections. The connecting capillary glass tubing is cut above the flask stopper, and the ends held together tightly by a flexible rubber connection; it is similarly connected to the gas burette. The rubber connections permit movement by the shaker mechanism. Each burette is surrounded by a Pyrex glass jacket such as that used on Liebig condensers. The jackets are connected in parallel to the constant temperature water bath and to a small rotary pump from a discarded automobile engine. The pump is operated by the same $\frac{1}{4}$-H. P. motor that operates the reaction-flask shaker device, the pump and motor shafts being directly connected.

The burettes are filled with distilled water and the lower ends connected by rubber tubing to a single leveling bottle. Pinch-cocks on each tube permit leveling of each burette individually by the one bottle.

Mercury is of such high density that a small difference in height in the leveling bottle and burette represents a relatively large volume of gas. Clear petroleum oil of low density (nujol) gave a poor meniscus because of distillation of moisture from the reaction flask. Distilled water previously allowed to stand in air several days was found to be very satisfactory.

In order to prevent growth of algae in the bath and in the water jackets of the burettes, a small amount of formaldehyde (about 1:1000) was added to the water in the bath.

Substrate

While it would have been desirable to use the volumetric apparatus without addition of an oxidizable substance, it was found that oxygen absorption was too small and inconsistent in the absence of such addition. Thus, 50 cc. of the freshly expressed apple juice absorbed less than 1 cc. of O_2 in 130 minutes. Peaches gave a similar result.

BUNZEL (**1, 2**), in his tests with the oxidase of potatoes and beet leaves, used pyrogallol as the substrate in his manometric apparatus. However, we found that the rate of absorption of O_2 by the pyrogallol was much greater at pH 5.7 in the absence of fruit juice than at pH 6.0 in juice. Evidently the fruit juice inhibited rather than catalyzed the reaction. At pH 10.3, non-enzymic oxidation was extremely rapid. Various tests gave additional evidence that pyrogallol, while probably satisfactory for potato

juice, is not very satisfactory for fruit juice. However, it proved useful with asparagus, spinach, string bean, and pea juices.[1]

Several of the commonly used colorimetric oxidase indicators, among them benzidine, hydroquinone, and guaiacol, were found to cause no significant additional O_2 absorption. Tannins and fruit coloring matter from several sources also were found of no value as substrates.

Since catechol tannins occur naturally in many fruits, and because ONSLOW (**10, 11, 12**) has reported that plant materials that give a positive test with guaiacum contain substances with a catechol grouping, this phenol was used in a number of trials with apple, apricot, avocado, olive, prune, peach, and pear juices. It was found to be a very satisfactory substrate. There was also only slight absorption of O_2 in water and in the boiled juice in the presence of the catechol, and absorption in these media ceased or became very slow after 15 minutes.

The CO_2 evolved by respiration of fresh fruit tissues and freshly expressed juice under the conditions of our tests was found to be appreciable, and it was evident that some means of absorbing this gas (such as that previously described) is necessary. The initial rate of absorption increased with increase in ratio of enzyme to juice but not in a strictly proportional manner.

Although the O_2 absorbed at the close of an experiment varied with the quantity of catechol initially present, the variation was not strictly proportional to the concentration of catechol. Thus when the quantity of catechol in one flask was 20 times that in another, the O_2 absorbed was less than twice that in the second. Possibly the reaction product inhibits enzymic action at higher concentrations of catechol. Two cc. of 5 per cent. catechol to 50 cc. of sample was found satisfactory.

Portions of apple juice were brought to various pH values ranging from pH 2.25 to 6.75 by addition of NaOH or of N/1 acid. The pH of the untreated juice was 4.0. The rates of gas absorption were determined at 25° C.

Reducing the pH value from 4.0 to 3.7 very greatly retarded O_2 absorption, and at 2.25–3.0 it practically ceased, being approximately the same as in boiled juice. Increasing the pH to 5.4 and 6.75 greatly increased the absorption of O_2.

Alkalinity naturally greatly favored O_2 absorption. The rate of absorption and the total amount of O_2 absorbed were much greater at pH 9, 10.1, and 10.8 than at pH 4.5; but much of this absorption is undoubtedly due to non-enzymic oxidation, as absorption is nearly as great in boiled as in unheated juices.

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[1] In tests made by H. M. PANCOAST.

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LEAF-WRINKLE, A NUTRITIONAL DISORDER OF SOY BEAN[1]

E. W. HOPKINS[2]

(WITH ONE FIGURE)

In the course of a study of the effects of light conditions upon nodule formation by soy beans, irregularity of development of the leaves formed after the plants were 3–4 weeks old was observed. This condition showed varying degrees of severity, from cases in which only a few plants were affected to others in which practically all plants were injured.

Injury occurs only in young leaves, appearing first in unopened leaves 5–10 mm. long. At first the hairs at the leaf tip become brown, followed by browning of the leaf tip, and later the edge of the leaf shows injury for perhaps a third of its length. The leaf does not die, but continues growth. The dead areas at the tip and adjacent sides prevent the blade from expanding and the leaf has a puckered appearance. Leaves which have opened and are 2–3 cm. long before the smallest leaves show injury will usually die at the edges, and will be nearly as wrinkled as the later leaves. Fully expanded leaves do not seem to be affected. In figure 1A are shown injured young leaves which have begun to expand, and in figure 1B, healthy young leaves.

Addition of boron as boric acid did not afford protection from injury to leaves subsequently formed after injury was first observed; nor could the suggestion that the symptoms of the disease resembled those of soy bean mosaic be confirmed. Injured leaves were rubbed on the upper surface of healthy leaves of various size without producing transmission of the injury. Evidence to be presented indicates that the injury is due to a nutritional disorder.

Injuries of similar nature produced by faulty mineral nutrition have been reported for plants other than soy beans (**2, 3**), so it seemed desirable to consider the possible rôle of the nutrient solutions in this case. The nutrient solution previously used was modified in several ways.

Manchu variety soy beans were planted out-of-doors in a bed of no. 3 quartz sand and transplanted after 6 days to 2-gal. pots of quartz sand. Until 16 days after planting, no nutrient solution was added to the pots, but the pots were set in shallow pans and watered daily with tap water. On the 16th day the pots were taken into a greenhouse and the nutrient treatments begun. A liter of nutrient solution was supplied daily to each pot, using the drip culture method (**4**).

[1] Contribution from the Hull Botanical Laboratory, University of Chicago.

[2] National Research Council Fellow.

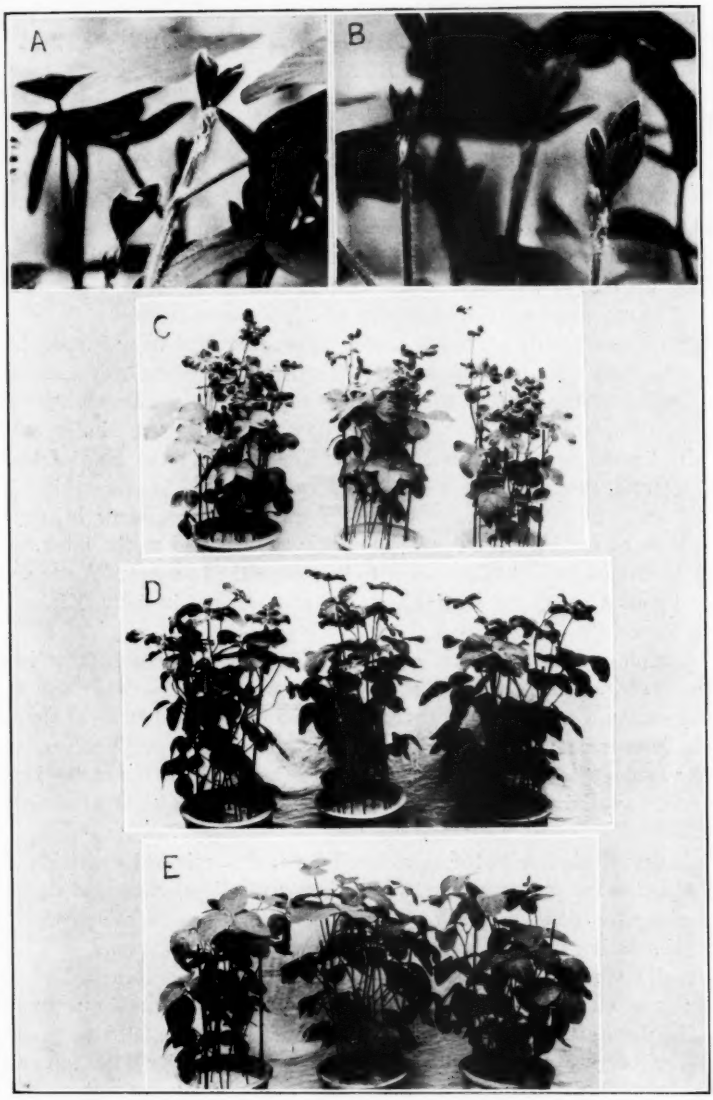

FIG. 1. A, B, young leaves of soy beans: A, leaf tips injured; B, leaf tips healthy. C, D, E, appearance of plants in three of the series: C, TIEDJENS and ROBBINS' solution + 30 mg. of NO_3 N per liter as potassium nitrate (severe injury to leaves); D, TIEDJENS and ROBBINS' solution + 30 mg. of NO_3 N per liter as calcium nitrate (very slight injury to leaves); E, BRYAN'S solution + 30 mg. of NO_3 N per liter as calcium nitrate (no injury to leaves).

Two basic nutrient solutions were used, BRYAN's (1) solution to which calcium nitrate was added to give 30 mg. of NO_3 N per liter of solution, and TIEDJENS and ROBBINS' (5) solution (series E, p. 12, modified by varying the nitrate) made up to contain 30 and 100 mg. per liter of solution of NO_3 N as potassium nitrate or calcium nitrate. The basic formula of either solution was that for a nitrogen-free nutrient, and the nitrogen was added in varying amounts as indicated. The nutrient solutions had the following formulas:

BRYAN's solution[3]		TIEDJENS and ROBBINS' solution	
K_2HPO_4	0.00143	KH_2PO_4	0.00633
$MgSO_4 \cdot 7H_2O$	0.00102	$MgSO_4 \cdot 7H_2O$	0.00237
$CaCl_2$	0.00225	$CaCl_2$	0.00146

The plants showed definite responses to the various nutrient treatments. In two of the treatments no injury to the young leaves was produced, and in the other three treatments injury occurred in differing degrees.

The plants receiving the BRYAN's solution or the TIEDJENS and ROBBINS' solution plus 100 mg. of NO_3 N as calcium nitrate were entirely free of affected leaf tips. Injury was most severe on the plants receiving nitrate as potassium nitrate. The group of plants given the TIEDJENS and ROBBINS' solution plus 30 mg. of NO_3 N as potassium nitrate showed injured leaf tips 5 days after beginning nutrient treatment, and after 15 days few leaves were free from injury. The plants at the age of 41 days are shown in figure 1C. The TIEDJENS and ROBBINS' solution plus 100 mg. of NO_3 N as potassium nitrate likewise produced dead leaf tips in 5 days, but after 15 days the number of injured leaves was considerably less than in the 30-mg. group.

Contrasted with the plants receiving potassium nitrate, those receiving calcium nitrate were relatively unaffected. On the plants given the TIEDJENS and ROBBINS' solution plus 30 mg. of NO_3 N as calcium nitrate, a few injured leaves were evident 15 days after treatment began (fig. 1D). Figure 1E shows the plants which were given BRYAN's solution. Table I indicates the composition of the nutrient solutions used (except for S and Cl) and the effect of these solutions on the leaf tips.

It is apparent from table I that the leaf-tip injury may not be ascribed to a particular cause on the basis of the present data. The difficulty does seem to arise from a complex relation of the various elements. If the nutrient solutions used in sets 1 and 2 be compared, it will be noted that if the N is increased there is less injury produced even though the amount of K present is considerably higher. Again, in sets 3 and 4, increase in N, in this case accompanied by an increase in Ca, results in no injury. In sets 4 and 5, where no injury occurred, the Ca/Mg ratio is about 4:1; and in

[3] The figures give the concentrations on the partial volume molar basis.

TABLE I

COMPOSITION OF THE NUTRIENT SOLUTIONS AND EFFECT ON PLANTS

TREATMENTS	K	Mg	P	Ca	N	RESULT ON PLANTS
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	
1. TIEDJENS and ROBBINS' +30 mg. NO_3 N as KNO_3	289	57	196	58	30	Severe injury
2. TIEDJENS and ROBBINS' +100 mg. NO_3 N as KNO_3	386	57	196	58	100	Less severe than in 1
3. TIEDJENS and ROBBINS' +30 mg. NO_3 N as $Ca(NO_3)_2$	247	57	196	101	30	Slight injury
4. TIEDJENS and ROBBINS' +100 mg. NO_3 N as $Ca(NO_3)_2$	247	57	196	201	100	No injury
5. BRYAN'S +30 mg. NO_3 N as $Ca(NO_3)_2$	112	25	44	90	30	No injury

set 3, where slight injury was found, the ratio is about 2:1. The most severe injury was produced by the solutions in which the Ca/Mg ratio was about 1. However, the addition of nitrogen was effective to a considerable extent in preventing injury (set 2). The ratio of K/Ca follows an order the reverse of that of Ca to Mg.

The leaf injury to soy beans discussed here appears to result from the relation of K, Ca, Mg, and N in the nutrient solution used. The conclusion seems warranted that with the TIEDJENS and ROBBINS' solution a low nitrogen level tends to produce injury to the plants.

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BRIEF PAPERS

THE TROPOGRAPH AND ITS USE[1]

(WITH ONE FIGURE)

A few years ago, in studying some of the forces exerted by plants, the writer devised certain equipment with which he measured the force which the petiole of *Podophyllum peltatum* develops in its response to the stimulus of gravity, as it is kept from bending. This apparatus was described at that time and was given the name tropograph.[2]

In recent studies of tropisms, the tropograph has been completely rebuilt, with many changes, although the general principles have been maintained. Before presenting the results of these studies it was thought desirable to describe in detail the tropograph and to indicate some of its uses.

This equipment is illustrated in figure 1. A chemical balance (*A*) is

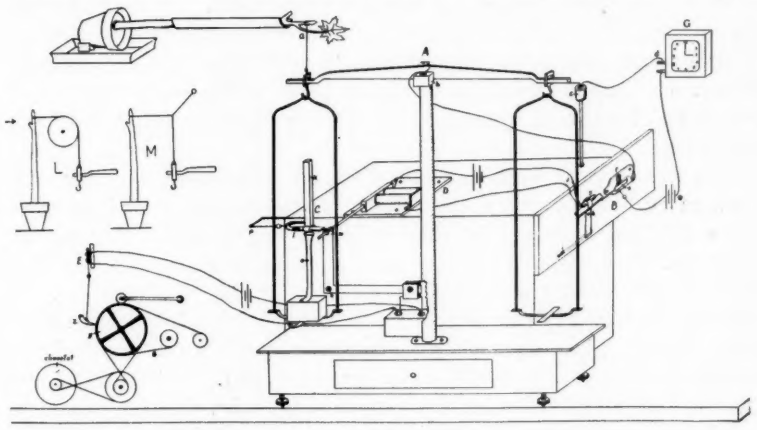

FIG. 1. The tropograph, an instrument for measuring the forces of tropic reactions.

used, and the end of the horizontal plant is connected by means of a thread or thin wire (*a*) to one end of the beam. To the other end of the balance is soldered a short piece of platinum wire which is curved so as to dip into a layer of oil covering the mercury in a mercury cup (*c*). By suitable weights in the pans of the balance, the latter is brought to equilibrium be-

[1] Contribution from the Department of Botany, University of Nebraska, no. 79.

[2] HIMMEL, W. J. A contribution to the biophysics of *Podophyllum* petioles. Bull. Torrey Bot. Club 54: 419–451. 1927.

fore the plant is attached. The end of the platinum wire and the mercury cup are so adjusted that, as the plant raises the opposite end of the beam only a fraction of a millimeter, the wire will come into contact with the mercury. An insulated wire connects the mercury with a clock (*G*) at *d*. The clock is so altered that it makes contact every minute between the wires leading into it at *d*. At the middle of the balance beam is also attached a short curved piece of platinum wire, by fastening it beneath the screw located at the top. This wire dips into a second mercury cup (*b*) so attached as not to interfere in the least with the freedom of movement of the balance. This wire may swing freely in the mercury as the beam moves. The other wire, connected with the mercury cup at *b*, connects with an electromagnet at *f*. An insulated wire connects this electromagnet with the clock through a set of two dry cells at *e*. When the plant, by pulling upward at *a*, makes contact at *c*, the clock completes the circuit and the electromagnet at *f* draws the armature (*x*) upward toward it.

To the left of this electromagnet (*f*) is a mercury switch (*g*) so mounted that it may swing freely on the pivot *h*. In operation this switch is nearly horizontal, but with the drop of mercury to the right. As the electromagnet operates, drawing the armature upward, the switch is tipped slightly the other way, causing the mercury drop to roll to the left, connecting the two wires at that end which lead away at *j*. A rubber band connecting the peg (*i*) and the base of the arm of the switch returns the switch to its original position.

The two wires at *j* connect with a second electromagnet (*D*) which is operated by a set of several dry cells. The electromagnet (*D*) operates a device (*C*) which drops steel balls, weighing approximately 1 gram each, into a container supported by the pan suspended from the left end of the beam of the balance and directly beneath the tip of the plant. The weights are located in tube (*k*), at the bottom of which is arranged a horizontal movable piece supporting the column of weights and attached to an extension of the armature of the electromagnet (*D*). This piece of metal slides in two lateral flanges at the bottom of the tube. When the mercury switch (*g*) closes the second circuit, the electromagnet (*D*) draws this piece at the bottom of the tube to the right and a steel ball falls through the hole in it at *l* which then is directly beneath tube (*k*). This movable base is curved so that the upper portion may move through a slit in the tube at the left and cut in between the two lowest balls, as the magnet (*D*) pulls it to the right. It thus supports the column of balls as the lowest one falls through the hole into tube *o* and on into the container on the balance pan. A rubber band attached at *p* helps pull the base back into its former position, thus permitting the column of weights to drop again on to the lower portion of the base so that another ball is in position to be released.

At *m* a light bar in the form of the letter **L** is attached to the extension of the arm of the electromagnet (*D*) and is pivoted at *n*. When the weight is dropped, the right end of this bar, which carries a wire shaped as an inverted **U**, is lowered so that the points of the wire dip into two mercury cups. This closes a third circuit in which a single dry cell and a third electromagnet are located, the latter operating an inking device at *z*. This inking device is continually describing a line on a strip of paper (*s*) which is carried around a revolving drum (*y*). When the electromagnet (*E*) operates, the inking device is drawn momentarily to one side, thus indicating when the weight is dropped. The drum is rotated on its axis in a clockwise direction by means of a clinostat to which it is belted through a couple of pulleys of different size, as indicated in the diagram, thus reducing the speed. On the top of the revolving drum (*y*) is a rubber roller which holds the paper against the drum. The rolls of paper used are the ordinary kind employed with adding machines. After the paper passes over the drum it is wrapped over a smaller cylinder which is rotated by a string wrapped about it at one end and which passes over an elevated pulley and is attached to a weight. This weight causes the cylinder to turn and keeps the paper taut.

Glycerin colored with a little gentian violet has been used for ink. This works very well, being just stiff enough to flow through the inking device at the proper speed and giving a clear, distinct mark. The inking device is a brass tube curved and tapered so that the opening at the tip is very small.

This equipment is equally suitable to the measurement of the force developed in response to light of various wave lengths and intensities, while the plant or plant part is not permitted to bend. When used in such studies, the plant is placed vertically and the thread connecting its tip with the balance beam is passed over a pulley as shown at *L* in the diagram. The tip of the vertical plant may also be connected to the end of the balance beam by means of a fine wire bent at right angles, and held in place by another wire connected at the apex of the angle in such manner that the pull by the plant in a horizontal direction will be equal to the upward pull on the balance. This is shown at *M* in the diagram.

The pot in which the plant has grown should stand or lie, as the case may be, in a tray as shown in the diagram, so that the plant may be supplied with water. This tray may be supported on a ring stand. The stem must also have some support for use as a fulcrum against which it may pull. The writer has used split glass tubing somewhat larger than the stem, and tied with linen thread around the hypocotyl or suitable internode of the stem. This may then be held in place by a burette clamp supported by a ring stand.

If less sturdy plants are being used, or if the force which the particular plant develops is not very great, a weight-dropping device adapted to small weights may be employed without altering any of the other parts of the tropograph.

By means of this apparatus the force which develops within the stem as it curves in response to the stimulus is automatically recorded. This force is equal at any minute to the sum of the weights in the container on the balance. The rate at which this force develops is thus recorded, as well as the maximum force which the stem is capable of exerting. The previous environment of the plant; that is, whether it has been in bright light or in the dark, will have a marked effect upon its response. This may be determined by the use of the tropograph. There seems to be a relationship between the food available within the plant and the force developed. The effect of the age of the plant, various gases, and chemicals upon the tropic force is also being investigated, as well as the relationship of the osmotic pressure of expressed sap to the tropic force as measured by the tropograph.

The principles employed here are not new, and have been used in the study of transpiration; but so far as the writer is aware they have not been employed in the study of tropic forces. It is hoped that these studies may contribute some information which will help in the more thorough understanding of the tropisms.—W. J. HIMMEL, *University of Nebraska, Lincoln, Nebraska.*

RELATIVE INFLUENCE OF NITRATE AND AMMONIACAL NITROGEN UPON INTAKE OF CALCIUM BY TOBACCO PLANTS

Recent investigations in the Soils Department greenhouse at the Connecticut Agricultural Experiment Station at New Haven showed that when tobacco was grown in sand and water cultures with varying combinations of nitrate[1] and ammoniacal[2] nitrogen, and with equal amounts of the most essential elements, the calcium intake by the plant was increased in direct proportion to increments of nitrate nitrogen over ammoniacal nitrogen supplied in the nutrient solutions.

The sand cultures in which quartz sand was used were carried on in 2-gallon glazed earthenware jars. Two-quart glazed earthenware jars, with thickly paraffined three-ply wood covers with holes in the center, were used for the water cultures.

The nutrient solutions used contained equal amounts of the most essen-

[1] Calcium nitrate.

[2] Ammonium sulphate.

tial elements. The variable was the carrier of nitrogen. Five solutions with different proportions of nitrate and ammoniacal nitrogen were employed with the sand cultures. Two solutions, one containing all nitrate nitrogen and the other with all nitrogen in the ammoniacal form, were used with the water cultures.

Havana Seed tobacco plants about 1 inch high were set in the sand and water cultures. One and one-half liters of one-third strength nutrient solution were added to each culture. At the end of ten days the solutions were renewed, and the renewal process was repeated at the end of the next seven days with one-half strength solution. Full strength solutions were then used for two periods of seven days each, after which they were renewed every three and one-half days until maturity of the plants.

Three successive crops of tobacco were grown in duplicate. During the early period of the tests the plants made more growth with increased proportion of ammoniacal nitrogen in the cultures, but later the nitrate end became larger and surpassed the ammonia end at maturity. The ammoniacal solutions produced blue-green while the nitrate solutions produced yellow-green colored foliage. During sunny days when the greenhouse became abnormally warm, the plants wilted progressively with increase of ammoniacal nitrogen while the nitrate cultures remained turgid. The plants in the cultures that contained all the nitrogen in the form of ammonia were stunted in growth and had rather thick, leathery leaves.

When the plants from the sand cultures were harvested, the roots were carefully examined. A form of root-rot increased in severity toward the ammonia end. It was most evident where all the nitrogen supplied was in the ammonia form. The same was the case in water cultures. The all-nitrate supplied culture produced healthy white roots while the culture that

TABLE I

TOTAL CALCIUM OF TOBACCO PLANTS GROWN IN SAND AND WATER CULTURES

PROPORTIONS $NO_3N : NH_3N$		SAND CULTURES IN PERCENTAGE OF Ca	WATER CULTURES IN PERCENTAGE OF Ca
		%	%
All	0	3.483	1.794
¾	¼	2.368	.....
½	½	1.449	.....
¼	¾	0.983	.....
0	All	0.652	0.618

received only ammoniacal nitrogen produced badly discolored and rotted roots.

Total calcium analyses were made of the plant material from all the cultures for the three crops. An average of these analyses is shown in table I.

The analyses (table I) show definitely that an increased proportion of nitrate nitrogen over ammoniacal nitrogen resulted in a larger percentage of total calcium in the plant material.

The root-rot phase of the investigation is being intensively studied in cooperation with the Tobacco Substation at Windsor, Connecticut.—H. G. M. JACOBSON, *Agricultural Experiment Station, New Haven, Connecticut*, and T. R. SWANBACK, *Tobacco Substation, Windsor, Connecticut.*

NOTES

Atlantic City Meeting.—Main features of the annual meeting were the incessant rain, the SACHS centennial celebration, and the annual dinner at which announcements of the award of the eighth life-membership and the third STEPHEN HALES prize were made. The annual dinner was also made the occasion of the reading of the second STEPHEN HALES address by Dr. W. W. GARNER.

The SACHS centennial was held in the Rose Room at the Traymore Hotel on December 28, 1932. A large gathering enjoyed the three addresses by Dr. D. H. CAMPBELL, of Stanford, Dr. R. H. TRUE, of Pennsylvania, and Dr. C. E. ALLEN, of Wisconsin.

In view of the small attendance at the banquet, it seems to be a mistake to place this function at the end of the meeting. It is suggested that the night preceding the Botanical Society dinner should be used by all other societies in the botanical field for annual dinners, so that all botanists may be free to attend the Botanical Society banquet. It would also be an advantage if tickets were prepared in advance of the meeting, and were placed on sale at the registration desk, and at all sessions of the Society.

Stephen Hales Prize.—The third award of the STEPHEN HALES prize was made to Dr. HUBERT BRADFORD VICKERY, of the Connecticut Agricultural Experiment Station, for his work on vegetable proteins. Dr. VICKERY was born at Yarmouth, Nova Scotia, February 28, 1893. He graduated with the B.S. and M.S. degrees from Dalhousie University, Halifax, in 1915 and 1918. He holds the Ph.D. degree from Yale in 1922. His early professional work was done in the high schools and normal college of Nova Scotia. During the last eleven years he has been research chemist at the Connecticut Station, and lecturer at Yale University since 1924. He has contributed a number of valuable papers on protein chemistry, and the constitution of the proteins of green plants.

Summer Meeting.—The meeting of the American Society of Plant Physiologists in June will be in connection with the A.A.A.S. World's Fair meeting. The meetings will be mainly joint meetings with other groups on June 20 and 21, with a special meeting of plant physiologists on Thursday morning, June 22. This special meeting will be a symposium on Radiation and Plant Life. The headquarters hotel for Section G and its affiliated societies is the Stevens Hotel. Early reservations should be made, as space will be limited by public demand. All meetings are being arranged to allow much freedom for visiting the exhibits at the Fair without missing important programs.

Support of Publication.—It has been necessary to defer a number of papers that were intended to appear in January and April, 1933, to later issues because of slow payment or non-payment of dues and renewal of subscriptions. It is impossible to use papers faster than our income allows, or to print a larger volume than our total finances will cover. This is a matter of importance to every member, and we hope that efforts will be made to maintain a stable income for PLANT PHYSIOLOGY. It is the only hope we have for continuance of the present generous-sized volume. The most serious consequence of reduced income is the necessity of holding all manuscripts for more than a year before they can be used. Papers intended for publication might as well be held for another year's work, and revised downward in size in addition.

Plant Physiology Laboratory at Purdue University.—The Department of Biology at Purdue University has had the good fortune to secure a new laboratory for plant physiology during the last year. Construction was effected during the summer and fall of 1932. The main laboratory is used for the introductory work in plant physiology. It is conveniently located adjacent to the physiology greenhouse, which is equipped with temperature control. A graduate laboratory provides facilities for graduate class work and research. A private research laboratory and office, and a centrally located stock room are also on the main floor.

An extensive basement has been constructed, and will be developed as funds become available. Plans for the basement include an oven room, a uniform temperature laboratory, a machine and work room, and adjacent light and dark rooms designed for plant growth experiments. An invitation is extended to plant physiologists to visit the laboratory when in Lafayette. —R. E. GIRTON.

Chemical Methods Committee.—This committee has under consideration the preparation of a supplementary report on chemical methods of analysis for plant materials. Dr. W. E. TOTTINGHAM, chairman of the committee would appreciate suggestions from members of the society as to matters which should be given attention in such a supplementary statement. He would be greatly assisted in this work if those who have had experience with new methods, or successes or failure with old methods would communicate with him at an early date.

Errata.—An unfortunate error was made in the notice accorded to Anthokinetics, by G. W. GOLDSMITH and A. L. HAFENRICHTER in the October, 1932, number of PLANT PHYSIOLOGY. On page 753, line 23, "heteranthous and ephemeral-heteranthous" should read "hemeranthous and ephemeral-hemeranthous."

On page 752, line 25, in the same number, for 400 read 4000. The editor regrets that these errors were not discovered in the proofs. Subscribers may find it useful to record the corrections where the errors occur to prevent future mistakes.

Root Nodule Bacteria.—A splendid monograph on root nodule bacteria and leguminous plants has been written by Dr. E. B. FRED, Dr. IRA L. BALDWIN, and ELIZABETH MCCOY. It is published by the University of Wisconsin as Studies in Science no. 5. It draws together the literature on this subject in masterly fashion. The approach is historical, and the background of present knowledge is skilfully arrayed. In the main part of the work the methods of isolation and study are presented, from which proceeds our knowledge of the morphology, life cycle, cultural and biochemical characteristics, longevity and species relationships of the organisms. The formation, histology and cytology of the nodules, mutual relationships between legumes and the bacteria, factors that influence nodulation, and natural and artificial inoculation are considered in later chapters. It is difficult to do justice to a work of this kind in a brief notice. There are 46 plates, a few charts, and almost a thousand citations. One can see that it will be of priceless value to other workers in this field, and that it will stand as a milestone of progress in the history of legume research. It may be obtained for $3.00 from the University of Wisconsin, Madison, Wisconsin.

Progress of Botany.—The first volume of an annual series devoted to Fortschritte der Botanik has been published by Julius Springer, 23–24 Linkstrasse, Berlin W9. The editor is FRITZ VON WETTSTEIN, of Munich. The progress in morphology, systematic botany, physiology and ecology is delineated for sixteen subdivisions of these fields by sixteen collaborating authors. The publication of research has become so voluminous that summaries are essential to proper perspective. The value of such a work depends upon the breadth of view, experience, and judgment of the individual collaborators. This volume for the year 1931 is a good beginning. One can find in a compass of 15 to 20 pages the main advances made during the year in the particular fields of botany included. The sketches are very helpful, even if they are condensed. On the whole they represent a satisfactory picture of the advances made during the year. The price quoted for this volume is 18.8 R.M.

Laboratory Manual of Plant Physiology.—The second volume of Das kleine pflanzenphysiologische Praktikum by L. BRAUNER, has been published by G. Fischer, Jena. (See PLANT PHYSIOL. 5: 291–292. 1930, for notice of vol. I). The experiments outlined in this volume deal with physico-chemical phenomena of cells. There are 9 experiments on diffusion phenomena,

26 on osmotic action, 28 on permeability, and absorption, and 20 on imbibitional swelling. The outlines are clear, the directions adequate, and the experiments well chosen to demonstrate principles. There are 61 text figures, and a brief appendix with some useful data tables, and directions for some simple technical operations in preparing apparatus. The price in paper covers is 5.5 R.M., 7 R.M. in cloth binding. Orders may be sent direct to the publishers.

Tribonema.—A brief monograph by EVA HAWLITSCHKA gives an account of this genus of heterokont algae (Die heterokonten Gattung *Tribonema*). The author gives the nomenclatural history of the genus, the present known geographic distribution, the results of artificial culturing in solutions and on agar media, methods of reproduction, vegetative (resting spores, aplanospores, zoospores) and sexual, cytological observations, and a discussion of the known species. It is a 36-page brochure with 18 figures. The price is 3 R.M. It can be ordered from the publisher, G. Fischer, Jena.